THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. LXXXVIII—No. 2

Issued March 1, 1929

BALTIMORE, U. S. A.

1929

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

Made in United States of America

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 88 MARCH 1, 1929 No. 2

THE INFLUENCE OF SUPRARENAL CORTEX AND MEDULLA ON THE GROWTH AND MATURITY OF YOUNG (WHITE LEGHORN) CHICKS

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Received for publication August 3, 1928

Fifty white leghorn chicks, when 12 days old, were divided into three groups and placed under experiment for eleven weeks to determine the influence of feeding desiccated suprarenal medulla and cortex, in addition to a standard or balanced ration. The first group, designated as lot I, consisting of 17 chicks, was used as a control. These were fed on a diet of Conkey's buttermilk chick feed, grit, cod liver oil and lettuce, and given free access to water. Each of the second group of 17 chicks, lot II, received daily in addition to the above diet and water, ½ grain of desiccated suprarenal medulla, fed in capsule form. The capsule was readily picked up and swallowed by the chick after the first few feedings. Likewise, lot III, 16 chicks, were each fed ½ grain of suprarenal cortex daily.

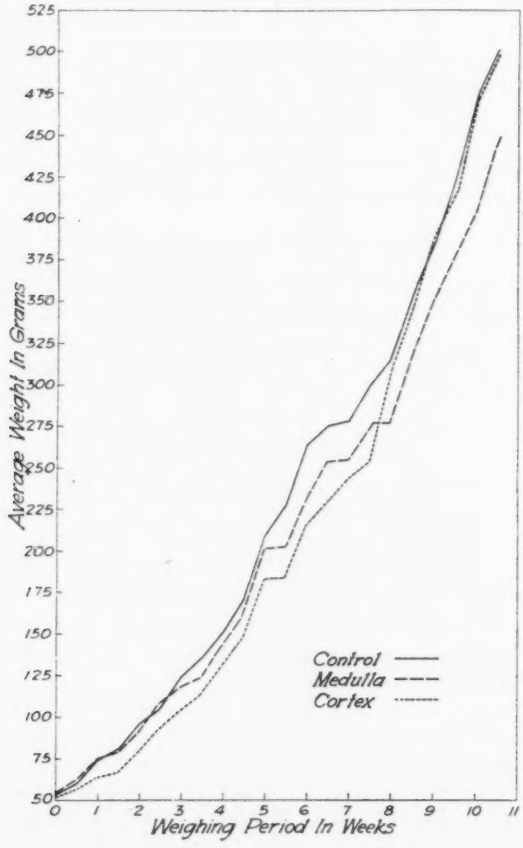
Each fowl of all three groups was weighed separately twice a week. Individual records were kept of weight, growth and appearance, and the results were tabulated. The graph compares the average weights of the three groups for the eleven-week period.

OBSERVATIONS. *Growth and general appearance.* Lot II, chicks fed desiccated suprarenal medulla, showed little variation in growth and weight from that of the control lot I for the first three weeks of experimental feeding. After that time and throughout the remainder of the experimental period, the growth and weight of lot II were retarded until finally a difference of 53.75 grams existed between the average weight of this group and that of the control. The difference in appearance of the two groups was even more striking than the figures indicate. The chicks fed

[1] Approved for publication by the Director of the West Virginia Experiment Station as Scientific Paper No. 67. Subsidized by the Purnell Fund and the G. W. Carnrick Company.

on medulla were smaller, more excitable and the feathers had a scraggly appearance.

Lot III, chicks fed suprarenal cortex, grew very slowly at first, their average weight falling below the average of the control group. At the beginning of the seventh week of experimental feeding, however, their average weight curve showed an acceleration and by the end of the eighth



week they were gaining faster than the controls in weight, having regained the loss noted in the first few weeks. The chicks fed suprarenal cortex were more lanky than the controls, and in length of limb, etc., had the appearance of being much larger and less sturdy.

Maturity. At the end of the eleven weeks of experimental feeding the chicks were killed and the testes removed, examined and weighed. The

average weight of the testes of the lot receiving desiccated suprarenal medulla was 0.1242 gram (5 males); the average of those receiving cortex was 0.2171 gram (7 males); while that of the controls was 0.2000 gram (6 males). If the weights of the testes are any indication of maturity, males receiving desiccated suprarenal cortex were more mature than either of the other lots, and those receiving desiccated medulla were less mature.

DISCUSSION. *Growth.* Chidester (1912) observed that the body weight of fowls decreased during a 10-day feeding with desiccated whole adrenal gland. It would appear that the medullary portion produced the loss in weight previously noted; however, Hogben (1922) produced a slight but significant retardation in weight in the case of birds injected with suprarenal cortex extract.

That suprarenal cortex feeding stimulates fat and calcium metabolism in rats was claimed by Hewer (1922).

A marked gastric disturbance in man, dogs and pigeons has been observed as a result of the oral ingestion of whole adrenal gland. (Rowntree, 1924; Hitchcock 1924; Barlow, 1926.) If this disturbance is due entirely to the medullary portion, which has not been definitely established, the gradual loss in weight of the chicks fed desiccated medulla might be accounted for in that manner. It appears from our experiment that the cortex is not concerned in these deleterious effects. Epinephrin produces similar gastric disturbance, loss of weight, etc. It should be expected that the medullary portion, rich in the alkaloid, would produce a physiological reaction similar to the extract.

McKinley and Fisher (1926) with white rats and Barlow (1926) with pigeons have shown a toxic reaction to whole adrenal gland administered by mouth. Feeding separate portions of the gland showed no toxic effect in our experiment with chicks, possibly due to the small amount fed daily.

Castaldi (1926) reports that adrenal cortex of beef, when fed to very young guinea pigs, produced an increase in body proportions and weight, the increase pertaining to muscles, fat and skeleton. An improvement in general condition is noted. This verifies the findings of Hewer (1922) noted above.

The same results have been obtained more recently by Fieschi (1927) with rabbits.

Reproduction. In 1916 R. G. and A. D. Hoskins reported that the testes of white rats fed on whole adrenal gland showed hypertrophy. This condition was probably due to the cortical portion of the gland.

Vincent (1917) stated that the cortex is derived from the germ epithelium and there is considerable evidence that it has important functions in connection with the development of the reproductive organs. He noted also that feeding young animals with adrenal gland substance seemed to stimulate the growth of the testes.

Hewer (1922) found that genital development was hastened in the male

gonads of rats by cortex feeding, but later degeneration of the seminiferous tubules occurred.

McKinley and Fisher (1926) noted that the testes of *cortex* fed white *rats* were 21.5 per cent heavier than the *testes* of the control animals. This percentage is *greater* than that obtained between the corresponding two groups of *chicks* in *our* experiment. The most striking contrast exists here between the group fed on *medulla* and the control group, the testes of the controls being 37 per cent heavier than the testes of those fed medulla. Evidently, medulla feeding caused a decided retardation in the maturation of the testes, whereas suprarenal cortex slightly accelerated their growth.

These experiments indicate the direct action on metabolism and maturity of the products of the two portions of the suprarenal gland. Since they produce such diverse results, it is evident that there are two distinct hormones whose action may be largely antagonistic, or, when the gland is acting normally, compensating.

SUMMARY

The influence of adrenal cortex and medulla on the growth and maturity of young (white leghorn) chicks is discussed.

1. Chicks fed desiccated suprarenal medulla grew almost the same as the controls for the first three weeks. After that time their growth was less rapid than the controls.

2. Chicks fed desiccated suprarenal cortex grew much more slowly at first than the controls. But by the end of the first eight weeks they began to grow more rapidly, and toward the end of the experiment almost equalled the controls in weight.

3. If the weights of the testes are any indication of maturity, males receiving desiccated suprarenal cortex were more mature than either of the other lots, and those receiving desiccated medulla were less mature than those of the other lots. The average weight of the testes of the lot receiving desiccated suprarenal cortex was 0.2171 gram; the average of those receiving medulla was 0.1242 gram; while that of the controls was 0.2000 gram.

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THE INFLUENCE OF DESICCATED SUPRARENAL CORTEX AND MEDULLA ON THE GROWTH AND MATURITY OF YOUNG RATS

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Received for publication August 3, 1928

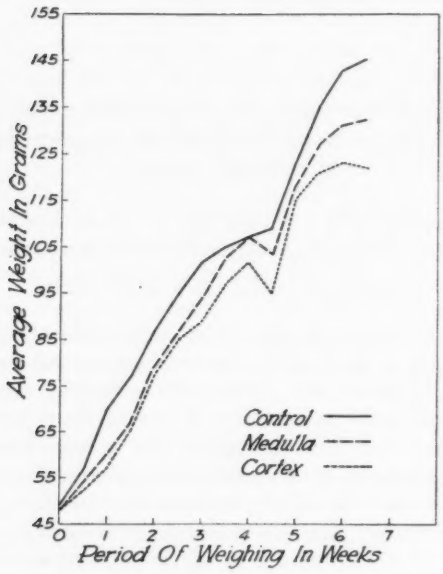
The experiment was undertaken to learn the effect of feeding young rats a small amount of desiccated suprarenal cortex and medulla together with a perfectly balanced diet. Most of the feeding experiments done previously with this gland product were done with the object of proving its effect in connection with diets deficient in one or more respects.

The diet used consisted of two parts of stone-ground whole wheat and one part of powdered milk (Klim, supplied by Merrill-Soule Company). This food was fed to the animals in glass cups in as large quantities as they would consume. A record was kept of the amount consumed by each animal. In addition to the regular diet one lot received 0.5 gram desiccated suprarenal medulla for each animal, mixed with a small amount of the regular diet. The larger food cups were removed until the mixture of medulla and food was consumed. The third lot received 0.5 gram of desiccated suprarenal cortex per animal fed in the same way. Distilled water was kept constantly before all of the animals. The animals were kept in individual round cages similar to those used by Sherman. Rats were weighed each Tuesday and Friday morning.

Stock. The animals were all from two litters of agouti rats and were placed on experiment when approximately one month old. Mothers of the animals had received the whole-wheat powdered milk diet as regular stock diet. *Five* animals were placed in each lot so that the number of males and females was as nearly equal as possible.

RESULTS. Growth curves show that both desiccated suprarenal medulla and cortex retard the growth of the young animals, the cortex retarding growth to a greater degree than the medulla. At the end of seven weeks the animals of each lot were transferred to a large cage for mating and the feeding of the gland products discontinued, so that the age of maturity might be tested.

[1] Approved for publication by the Director of the West Virginia Experiment Station as Scientific Paper No. 76. Subsidized by the Purnell Fund and the Merrill-Soule Company.



LITTER RECORDS

All controls placed together September 23, 1927

Subject	*Litter date*	*Number of young*
No. 1, female. Died 9/28/27		
No. 3, female	(First) November 16, 1927	5 young
	(Second) January 7, 1927	5 young

Group fed on medulla placed together September 23, 1927

No. 7, female	November 26, 1927	8 young (died)
No. 9, female	December 19, 1927	7 young

Group fed on cortex placed together September 23, 1927

No. 11, female	(First) November 2, 1927	3 young (died)
	(Second) December 11, 1927	4 young
No. 12, female	No litter	
No. 14, female	November 26, 1927	6 young
No. 15, female	(First) October 17, 1927	4 young (died)
	(Second) November 18, 1927	5 young

Females 11 and 15, both fed on suprarenal cortex, were the first to produce litters. No. 15 gave birth to 4 young, 24 days after possible copulation, and no. 11 gave birth to 3 young, 40 days after being placed in the mating cage. The first litter to be born to a control female was not produced until 54 days after possible copulation, which was 10 days sooner than the date of the first litter produced by a female fed on medulla. No. 1,

a control female, died 5 days after being placed in the mating cage; No. 12, another female fed on cortex, never reproduced.

It appears that the maturity of young female rats is hastened by suprarenal cortex feeding. This effect is not produced by suprarenal medulla.

DISCUSSION OF LITERATURE. *a. Growth.* Chidester (1912) observed that the body weight of fowls decreased when fed on whole suprarenal gland.

Hogben (1922) showed a retardation in growth of fowls by successive injections of suprarenal cortex.

Other experiments by Hewer (1922) with rats; McKinley and Fisher (1926) with rats; Castaldi (1926) with guinea pigs; and Fieschi (1927) with rabbits, have shown that the retardation previously reported was not due to the cortex, these authors reporting an increase in weight when cortex was fed.

Our results with rats in this experiment show, on the contrary, a decided decrease in weight both with the medulla and with the cortex.

Hitchcock, (1924), Rowntree (1924) and Barlow (1926) have shown that there is a disturbance of gastric functions as a result of the oral ingestion of whole adrenal substance, especially in the case of the medulla. It would appear from this experiment that cortex might cause gastric disturbance in rats even more than medulla, otherwise the decided retardation in weight could hardly be accounted for.

b. Reproduction. R. G. and A. D. Hoskins (1916), Vincent (1917), Hewer (1922) and McKinley and Fisher (1926) have all shown that hypertrophy of the testes accompanies the feeding of suprarenal cortex in rats. The litter records shown above indicate that the age of maturity is hastened. This result would be expected in view of the fact that suprarenal cortex feeding increases the size of the testes.

The feeding of suprarenal medulla has little, if any, effect on the age of maturity in rats; although it has been shown by the authors (in press) in an experiment on chicks that suprarenal medulla causes an inhibition in the development of the testes.

SUMMARY

1. Growth of young rats is retarded by 0.5 gram of either desiccated suprarenal medulla or cortex and is retarded, especially at first, by desiccated suprarenal cortex.

2. Young female rats fed 0.5 gram of desiccated suprarenal cortex (as shown by earlier litters) reach sexual maturity sooner than controls or those fed on 0.5 gram desiccated suprarenal medulla daily.

Males were not tested independently with older females so that we are not certain whether or not they reached sexual maturity earlier than the females of their own litter.

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THE EFFECTS OF CARDIAC SYMPATHETIC NERVES UPON HEART BLOCK

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Received for publication September 29, 1928

The immediate object of this investigation was to determine the effect of the sympathetic nerves upon heart block induced by clamping different regions of the turtle heart. The results of such an inquiry are essential to the interpretation of the reported effects of stimulation of the mixed vagus trunk upon heart block. That stimulation of the vagus can increase the degree of pathological and experimental auriculo-ventricular block is well established both for mammals and for cold-blooded animals. Lewis and Drury (1923) and Drury (1925) find, however, that when partial block is produced in the auricular tissue of mammals either by clamping or by cold, vagus stimulation invariably relieves the block; and Garrey (1925) has reported a dual effect in the cold-blooded heart (turtles) when the mixed vagus trunk is stimulated. Usually the degree of block is increased, rarely relieved, and sometimes the two effects are obtained in sequence.

Such recovery from block was regarded by Lewis and Drury to be a "reversed" vagus action such as was first described by Gaskell in his earlier papers (1882, 1883 and 1884), although in a later paper (1886) Gaskell attributes these effects to the action of sympathetic fibers unavoidably included in its stimulation. He states that the reverse effects occur when the action of the sympathetic is at its maximum and that there is "undoubted evidence that the sympathetic is able to restore the sequence between auricle and ventricle." Garrey (1912, 1925) is in consonance with this view when he suggests that the relief of block upon stimulation of the turtle's vagus may be due to the sympathetic fibers. In addition, there is anatomical (Billingsley and Ranson, 1918), histological (Iwama, 1925), and physiological evidence in support of this suggestion. It thus becomes desirable to investigate specifically the action of *sympathetic* effects upon heart block in the turtle. This is especially necessary since Lewis and Drury find an increase in auriculo-ventricular block with a decrease in auriculo-auricular block—a paradoxical phenomenon—and they emphasize the striking fact that the latter result disappears when under the influence of atropin.

[1] Fellow of the Rockefeller Foundation.

METHODS. About twenty turtles of the species *Pseudemys scripta*, *Pseudemys elegans*, *Pseudemys troosti* and *Emys blandingii* were used for these experiments.

After the animal was pithed, the plastron was dissected off and two triangular portions were cut from the margin of the carapace at the level of the anterior limbs, so that these could be completely stretched in fixing the animal upon the board. By this means the lateral sympathetic chains, deeply placed in the axillary region, were made more easily accessible.

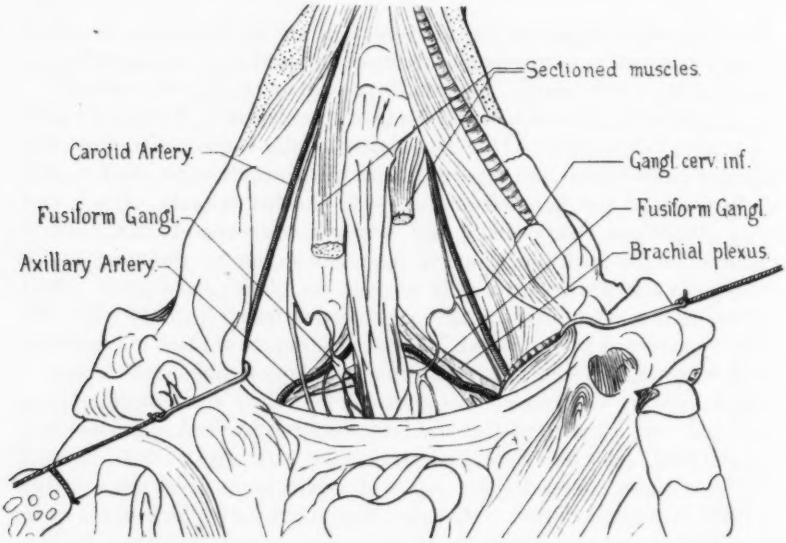

Fig. 1. General arrangement and relations of cardiac sympathetic nerves in *Emys blandingii*, after preparation for stimulation as described in text.

After preliminary dissections and stimulation experiments and perusal of previous work on the subject (Gaskell and Gadow, 1884; Kazem-Beck, 1888; Dogiel and Archangelsky, 1906), it was decided that the cardiac sympathetic fibers were most satisfactorily stimulated by applying electrodes to the so-called *fusiform ganglion*—the equivalent of the stellate ganglion of mammals. This ganglion is to be found in the axillary region, taking as points of reference for its location the brachial plexus and the axillary and vertebral arteries (see fig. 1). Great care must be exercised to avoid stretching the very delicate fibers or causing hemorrhage which would obscure the field of operation. Experience indicated that the

easiest and safest way of exposing this ganglion, after pulling aside the superficial muscles, was to transect the bands formed at both sides of the vertebral column by the long neck muscles, a little above the level of the brachial plexus. Their ends retract notably (as the figure shows) and allow free and easy access to the ganglion which then can be delicately isolated without injury or loss of blood.

After the nerves were prepared, the pericardium was opened, the heart exposed, and two convenient points, selected according to the experiment to be performed, were connected by the Engelmann suspension method with light writing levers. A modified Gaskell clamp, with a micrometric screw of 20 threads per centimeter and jaws protected with pure nitrommeter rubber tubing to assure a delicate and progressive compression with minimal injury of the tissues, was very slowly applied at a point in between until the desired degree of block was obtained. In any case stimulation was not performed until the secured degree of block had persisted for a reasonable time—sufficient to assure its uniformity and stability throughout the approximate duration of the experiment. Experience corroborates Gaskell's statement made in 1883, that a partial block "lasts sometimes a long time without alteration, at other times lasts only a short time and then every contraction passes over." For this reason patience was employed each time, until uniform block conditions were established, to avoid taking as an experimental improvement of conduction what only might have been a "spontaneous" chance recovery.

From the early investigators in this field it is also well known that drying increases the block while remoistening removes it. If changes of this nature pass unnoticed, that which is only a chance removal under its influence may be taken for a nervous action. Great care was exercised, therefore, to keep the heart surface frequently bathed with its own pericardial fluid and with lymph applied to the heart with a dropper.

The experiments were made with the clamp in the four following positions:

1. Transversely between the basal and middle third of the ventricle. As the ventricle was in some cases wider than the clamp jaws, it was necessary to make previously a slight lateral cut of the walls to narrow the ventricle to a bridge upon which the clamp could be easily applied. The bridge, however, was never as narrow as in Gaskell's old experiments (1883), and never produced block by itself. Gradually the clamp was tightened to induce the desired degree of *ventriculo-ventricular block.*

2. By sliding one of the clamp jaws behind the aorta with its edge upon the anterior auriculo-ventricular groove and by placing the other upon the dorsal side of the A-V junction, pressure was applied to underlying auricular tissue and different degrees of *auriculo-ventricular block* were obtained.

The experiments in these two first types of clamping were done after

exsanguinating the heart at the moment of applying the pressure—a method corresponding to that used by Garrey (1912) in his similar experiments upon the action of the vagi. Otherwise, as soon as the clamp is applied, the sinus and the incoming veins become extraordinarily dilated and place the organ in very abnormal conditions, evidenced together with other signs by a notably slow rate.

3. After cutting off the ventricle at the level of the auriculo-ventricular groove, the clamp jaws, held in a vertical position, were applied and tightened upon the middle line between the auricles, so that checking of impulses of the right auricle from the left resulted in an *auriculo-auricular block*.

4. Finally, with care not to stretch the veins going to the sinus venosus or the sinus itself, the clamp was applied between this segment and the right auricle, thus determining a *sino-auricular block*.

Once these steps were taken, the already dissected sympathetic ganglia were lifted upon fine platinum hook electrodes, the two wires of which were separated approximately by 1 mm. distance, protected upon their outer and lateral surfaces with a heavy coat of shellac to avoid diffusion of the faradic current used for stimulation. This was important because all spread of current which might determine confusing effects—especially through the vagus—was to be avoided. The neighboring nerves of the brachial plexus served very well as an index of the spread of current. If this spread occurred, violent movements of the corresponding limb, to the extent of disarranging the tracing levers, indicated the need for a better position of the electrodes. But if no such movements took place, it was certain that there was no spread of current to the more distant vagus. On this basis, none of the accompanying tracings can be suspected to be due to a vagus stimulation.

The length of the faradic stimulation (a Harvard Apparatus Company coil connected with two dry cells) was recorded with an electro-magnet signal in the primary circuit and its intensity expressed in centimeters of distance between the two coils. A Jacquet clock recorded the time at intervals of three seconds.

Before repeating an experiment or passing to work upon another heart segment (this was done very rarely), the clamp was removed, the writing levers disconnected and the heart allowed to rest without the compression or stretching forces, while the whole region was covered with a sheet of filter paper to provide a moist chamber, and the nerves were also removed from the electrodes. Very often, after such conditions were maintained for one or two hours, a subsequent experiment gave some of the best results obtained.

RESULTS. *Sympathetic effects upon ventriculo-ventricular (intraventricular) block.* In most of the turtles used the effects of sympathetic stimu-

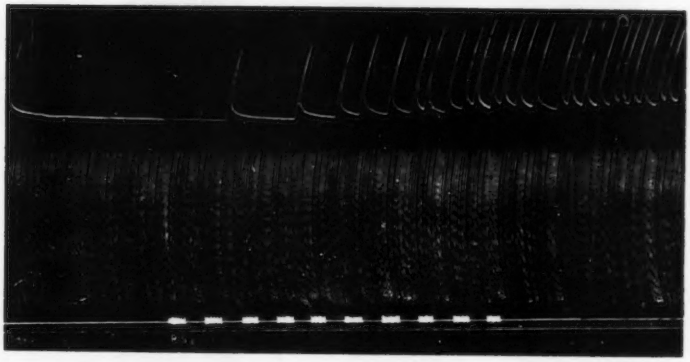

Fig. 2. Severe block, produced by clamping between the right auricle and the ventricle, is gradually relieved after the right fusiform ganglion is stimulated at 20-second intervals for periods of the same duration (coil distance = 5 cm.). *V.* = ventricle; *R. Au.* = right auricle; *R. S.* = right sympathetic; time here and in other tracings, 3 seconds

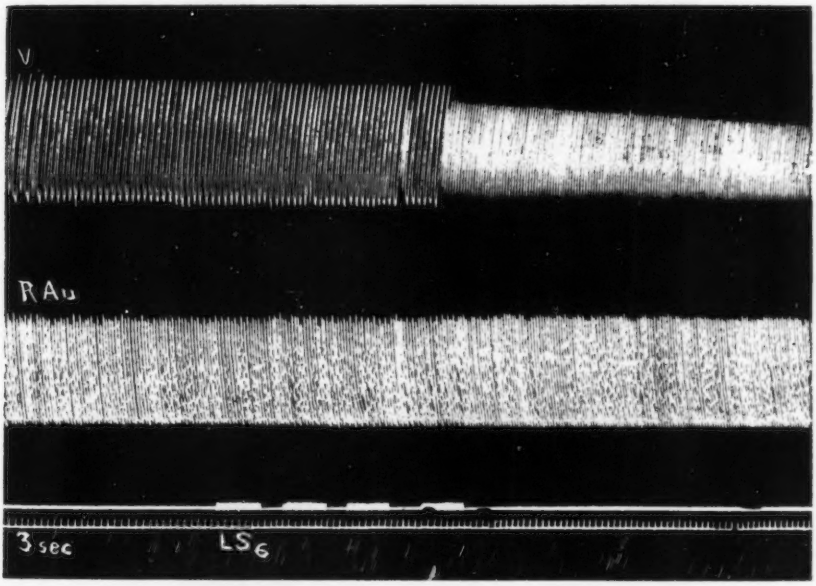

Fig. 3. Repeated 15-second stimulations (coil at 6 cm.) at 10-second intervals, accelerated the auricle from 40 to 47 beats per minute and restored the auriculoventricular rhythm from 2:1 to the normal 1:1 relation.

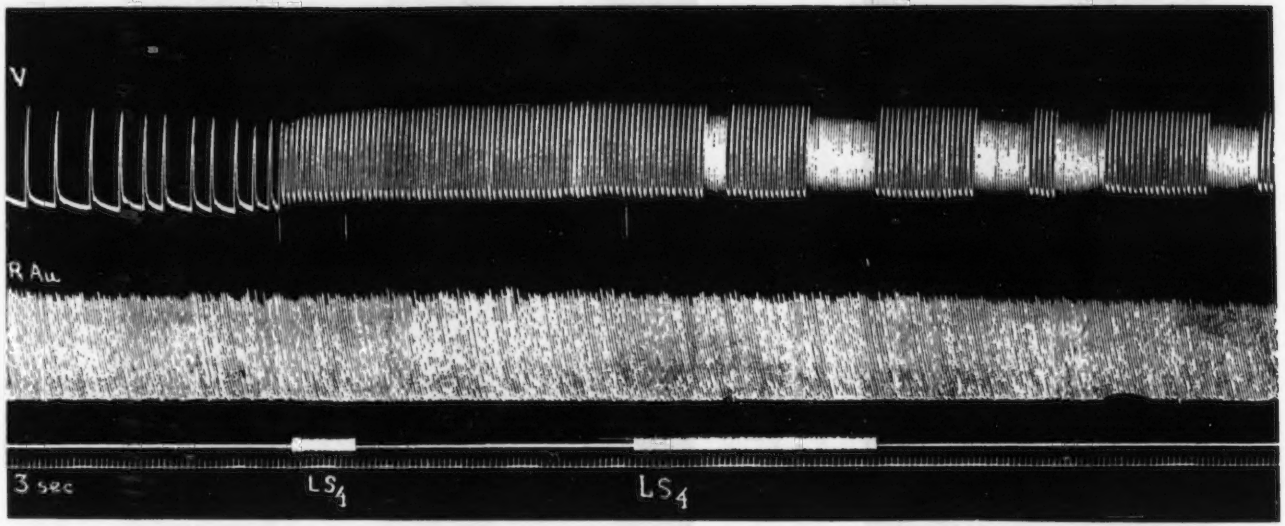

Fig. 4. Upon strong stimulation (coil at 4 cm.) of the left fusiform sympathetic ganglion, a severe block (7:1) was quickly reduced to 2:1. A second more prolonged similar stimulation brought periodically the ratios of contraction of the two cardiac divisions to 1:1. The auricle was not accelerated and continued beating 42 times per minute, but the ventricle accelerated first from 6 to 21 and finally to 42 per minute.

lation upon the conduction of impulses partially or completely blocked between the basal and the apical two-thirds of the ventricle were tested shortly after preparing the animal as well as after a period of rest. Strong,

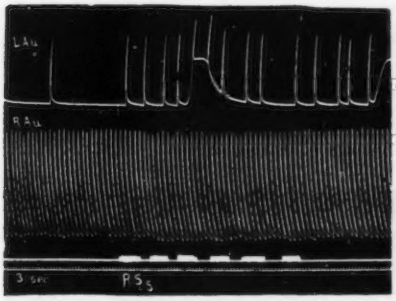

Fig. 5

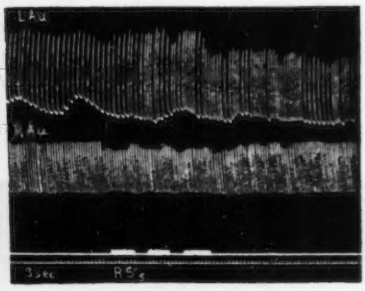

Fig. 6

Fig. 5. The first of a series of stimulations for 10 seconds at intervals of 10 to 15 seconds reduced a profound auriculo-aricular block of 15:1 (right auricle beating 14 times per minute) to 3:1. The effect continued for a long time. No changes in the frequency or amplitude of the right auricular beat occurred. *R. Au.* = right auricle; *L. Au.* = left auricle.

Fig. 6. Independently of any noticeable accelerator or augmentor effect upon the right auricle, strong sympathetic stimulation (coil at 5 cm.) of 15 seconds at 10-second intervals reduced for two minutes an auriculo-auricular block of 2:1 to 1:1. Then block reappeared.

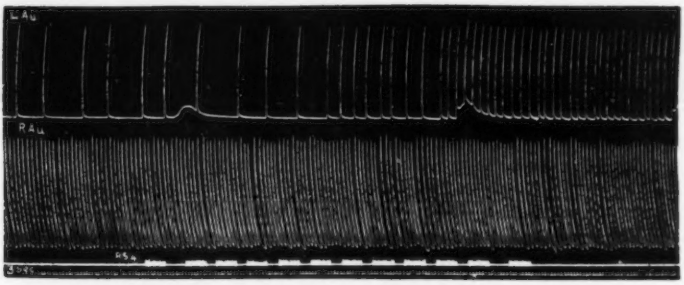

Fig. 7. A series of periodic strong stimulations of 20 seconds' duration (coil at 4 cm.) at approximately 10-second intervals, changed an irregular auriculo-auricular block of 4 or 6:1 into a more regular one of 2:1, independently of any important change in rate or frequency of the right auricle.

medium and weak stimuli were tried but in no instance was there any indication of either increase or decrease of the block, although the augmentor and accelerator effects were obvious. The ratio of auricular to ventricular beats remained unchanged.

Sympathetic effects upon auriculo-ventricular block. The variation of these effects is illustrated in figures 2, 3 and 4.

Figure 2 is very eloquent because it shows how a severe A-V block, established and maintained for some time, was gradually removed upon repeated stimulation of the right sympathetic cardiac nerves. The effect began after a latent period of about a minute after the first stimulation and did not seem further improved by additional similar stimulations.

Figure 3 shows how a 2:1 block was relieved after another fairly long period of stimulation (more than a minute), but the change took place

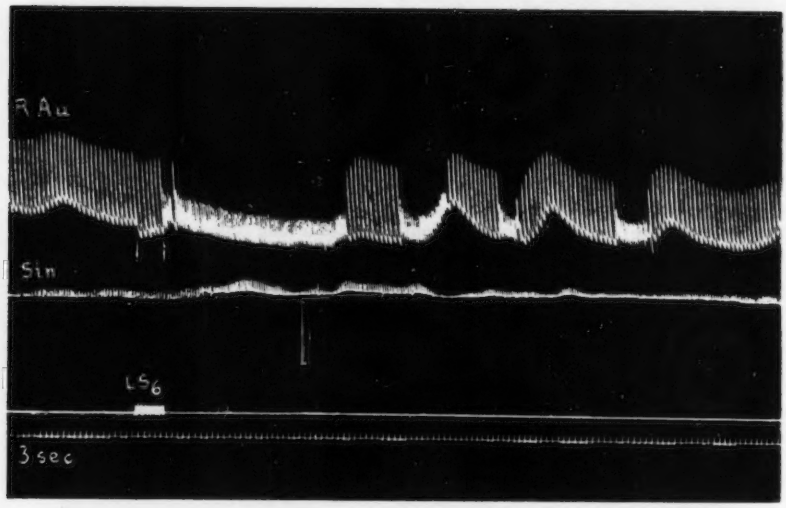

Fig. 8. A stimulus (coil at 6 cm.) is applied to the left fusiform ganglion until the sino-auricular block of 2:1 is relieved after 15 seconds of stimulation. The effect remains for over a minute and then the block gradually returns to its former value of 2:1, through sudden alternate periods of block and normal sequence independently of the tonus oscillations of the sinus. The augmentor effect of the sympathetic upon sinus contractions is clearly demonstrated.

quite abruptly though preceded by an indication that the change was going to occur. The effect lasted for several minutes.

Figure 4 is still more impressive because it illustrates how a stronger single stimulation of the left fusiform ganglion quickly reduced a profound block of 7:1 to a more superficial degree of 2:1, and how, upon repetition of this stimulation, after a long latent period there was reëstablishment of normal 1:1 rhythm.

It becomes clear after inspection of these tracings and their legends that they all needed strong stimuli to evoke the results, and were not necessarily

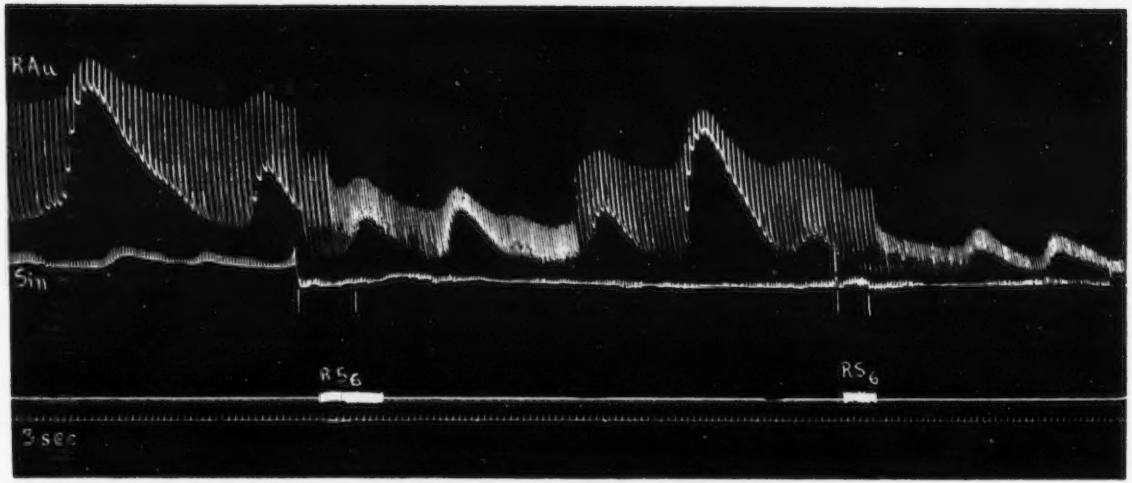

Fig. 9. A few seconds after beginning 15 seconds of stimulation (coil at 6 cm.) of the right fusiform ganglion, the sino-auricular block of 2:1 is relieved for nearly two minutes and then former conditions are reëstablished. After a rest of little more than two minutes, a new stimulation of 15 seconds brings about the same de-blockage, persisting about 2 minutes, and then a return to block was reached by alternative periods (as in fig. 8). The changes were not related to the tonus waves of the auricle.

linked with important changes in either the amplitude or rate of the auricular beats.

Sympathetic effects upon auriculo-auricular (intra-auricular) block. The different ways in which intra-auricular block is relieved upon sympathetic stimulation may be seen in figures 5, 6 and 7. The improvement of conduction may be determined just a few (10) seconds after stimulation begins and last for minutes afterward (fig. 5); it may come out irregularly after a longer latency and recede in about two minutes (fig. 6); or it may appear still more slowly, the conduction from right to left auricle improving gradually (fig. 7) and persisting for a long time.

Sympathetic effects upon sino-auricular block. Stimulations of both fusiform ganglia bring up to normality conduction depressed by clamping between sinus and right auricle, after a fairly average latent period of 15 seconds (see figs. 8 and 9). The effect takes place abruptly, and sometimes reappears periodically but in general it tends to disappear after some minutes.

Before summarizing these results, it is important to state that they are not obtained in 100 per cent of the experiments, but this has no particular significance since some inconstancy has been invariably found by innumerable investigators with relation to accelerator and augmentor effects of the cardiac sympathetic, and even in relation to the easily demonstrable and more constant effects of vagus stimulation upon the heart.

General remarks upon these results. The general features of these results coincide very fairly with those involving sympathetic stimulation. A strong faradic stimulus is needed, as it is also needed to evoke accelerator or augmentor effects; the reactions require a long period of latency and even may appear some time after stimulation is over. Frequently they need the repetition of short periods of stimulations at short intervals before their induction.

In a general way it may be stated that the nearer the blocked region was to the sinus the shorter was the duration of stimulation necessary for the decrease of block and the shorter was the period of latency, while no stimuli were effective in changing the character of block within the mass of the ventricular muscle. This suggests that the turtle's sympathetic must exert its action mainly upon the basal parts (sinus and auricles) of the heart, and recalls the old assumption of Gaskell (1886) that in the turtle and the crocodile it is almost if not entirely confined to the auricles. In addition, some of our experiments indicate that each sympathetic is more strongly related to the auricle of the same side. A few strong stimulations of the left fusiform ganglion (see fig. 10), although they did not improve conduction between auricles, determined a sharp augmentor effect upon the left one. Nevertheless, the cardiac fibers of both sides were

equally effective in bringing about the improvement of longitudinal conduction, and thus it is possible that the local effects were shown only when the clamp was too much on the right side.

The improvement of conduction exerted by the sympathetic appears not necessarily to be related to the accelerator or augmentor effects. These may exist without any improvement of conduction, which came later in some of our tracings, or may be lacking altogether. Again, this dissociation of effects has been also repeatedly shown in relation to augmentor and accelerator effects (Mills, 1884; Frédericq, 1913; etc.)

Concerning the cardiac tissue upon which the sympathetic exerts its action to improve conduction, the facts here presented do not allow one to make any important inferences. They could be interpreted as purely

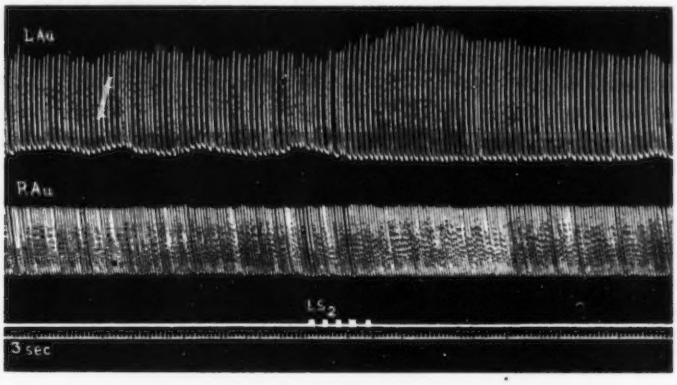

Fig. 10. A series of five strong stimulations (approximately of five seconds' duration at equal intervals, coil at 2 cm.) determined an augmentor effect, localized in the left auricle without exerting any similar effect on the right one, or modifying conduction between them.

nervous effects through the sympathetic fibers going through the compressed region, as it has been demonstrated by Garrey (1911) and Frédericq (1912) for the heart, and by Meek and Leaper (1911) for frog's sartorius nerves that nerve fibers are more resistant to compression than muscle. They could be also interpreted as a pure effect upon muscle. Lewis and Drury (1923) and Drury (1925) established blocks by applying pressure or cold between different parts of the dog's auricle and came to this conclusion, but it would be unsafe to extend this interpretation to other segments of the heart. Even in the turtle Laurens (1913, 1917) demonstrated an anatomically and functionally well differentiated conducting system, and Boeke (1926), who has carefully stained the fiber nerve endings in *Emys* and *Cyclamis* hearts, describes the existence of a real auriculo-

ventricular conduction system formed by elements suggesting the Purkinje fibers, each of which receives individual innervation.

The solution of this problem will be obtained only by more delicate methods than those used for this investigation. It will suffice at this juncture to show that *the effects described in this communication are characteristic of sympathetic action.*

Discussion. Evidence presented in this paper is entirely in accord with the experimental results of previous investigators. Frog, turtle and crocodile hearts, kept over night after death of the animal and found flaccid and apparently motionless, with only the sinus beating, were observed by Gaskell upon stimulation of the sympathetic to begin to beat, and soon the whole heart beat regularly and strongly, because "the action of the nerve is perhaps rather to improve the conduction power of the different muscular tissues which together form the heart, than actually excite into action any part of those tissues" (Gaskell, 1886). Indeed, it is well known that the process of dying of the heart is characterized by the formation of successive blocks to the progress of the wave from one division to another. Bayliss and Starling (1892) found that improvement of conduction across the A-V junction, due to stimulation of the accelerators, could be invariably demonstrated. Hunt (1899) noticed that the irregular sequence of beats that followed section of the accelerators, upon stimulation of these nerves, was replaced by a rhythm in which "the ventricle followed every auricular beat" (Hunt and Harrington, 1897). Dale and Mines (1913) showed that the effects of such stimulation were indicated on the electrocardiogram by a shortening of the A-V interval, and Routier (1915a), experimenting on dogs, came to the conclusion that there was a similar relation between sympathetic and conductivity. Adrenin action upon the heart is, as is well known, interpreted in the same sense (Gunn, 1914; Routier, 1915b; Danielopolu and Danulescu, 1916). Now, how do these results conform to those obtained after vagal stimulation? Do these nerves exert the same effect upon conduction? Do the vagi exert the effect of increasing conduction (Erlanger and Hirshfelder, 1906), or is the improvement sometimes observed the result of a variable degree of effectiveness of the excitation of sympathetic fibers mixed with the vagus nerve trunk?

One possible indirect effect of vagal stimulation, described by Garrey, in which the vagi may decrease the block is the condition in which their chronotropic effect is pronounced. In such conditions the "auricles are slowed sufficiently to permit a degree of recovery between contractions which more than neutralizes the negative inotropic and dromotropic changes" (Garrey, 1912). But when chronotropic effects of either vagus were little appreciable or when the nerves were excited intracranially with no participation of sympathetic, the pure vagal effect observed by this

author consisted in an increase of block. Indeed, the most acceptable idea is that the opposite vagal effect of improvement of conduction is rather bound to "different classes of nerves" (Heidenhain, 1882); to "alone visible" effects of the stimulation of sympathetic fibers (Gaskell, 1886), or more precisely "to masked accelerator fibers" in the vagus trunk (Dale, Laidlaw and Symons, 1910). Garrey (1925), after confirming more recently in nine different species of turtles that the usual effect of the vagus

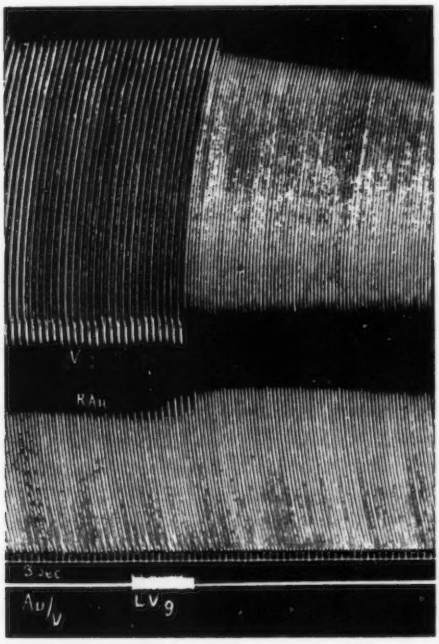

Fig. 11. One experiment of stimulating the left vagus (during 30 seconds, coil at 9 cm.) that caused a 2:1 A-V block to disappear, showing that in producing this effect slowing of the rate is not necessary. The block is not relieved until the augmentor effects are clear.

stimulation is to enhance the degree of block produced by a clamp which compresses auricular tissue, is in accord with this view.

Lewis, Drury and collaborators have published a series of papers advancing the idea that improved conduction is a true vagus effect. They do not accept the idea that local effects may result from a selective action of accelerator vagal fibers *"which might be supposed to exist"* (Drury, 1925). This obliges us to consider at once to what extent the existence of accelerator vagal fibers may be considered as a mere supposition.

Anatomically, Dogiel and Archangelski (1906) find sympathetic fibers rejoin the vagus below the inferior cervical ganglion, thus resulting in an appreciable thickening of the vagus below this point. Dissection confirms this finding constantly in *Emys blandingii* (see fig. 1). But of course this is not the only arrangement for it is not rare to find the vagus apparently entirely detached from the sympathetic in its lower part with confluence higher up. In such instances it is impossible anatomically to identify sympathetic fibers going to the heart. Nevertheless, on purely anatomical grounds a constant communication has been found between the superior cervical ganglion and the nodose ganglion of the vagus in the cat (Billingsley and Ranson, 1918), and histologically the descending path of part of the thus originated fibers has been traced out by Iwama (1925). Experimental evidence already quoted, as well as experiments that show that atropinizing or nicotinizing of the heart reverses the usual vagal effects, is, by itself, very significant and justifies the assertion that the presence of sympathetic descending fibers in the vagus trunk is more than a mere supposition.

The hypothesis of the vagal nature of the improvement of cardiac conduction is based primarily in one of the accompaniments of the phenomenon; namely, it is "accompanied by profound inhibitory action upon the other parts of the heart" thus described by Drury (1925): "while vagal stimulation produces a profound degree of block at the A-V junction, it quickly relieves the block previously produced at the base of the auricular appendix by a clamp recently applied." Garrey's tracings (1912) show very clearly that neither strong nor weak inhibition at the A-V junction is necessary to bring about the effect. On the other hand, it is difficult, though not impossible, to understand how the same nervous action would exhibit such opposite effects upon different segments of the heart. If the inhibitory effect is considered as due to genuine vagus inhibitory fibers, while improvement of conduction is due to sympathetic fibers, the results are in admirable harmony with the known physiological anatagonism of the sympathetic and parasympathetic fibers.

How then is it possible to explain the disappearance of the reaction after the intravenous injection of atropin (the second strong proof of Drury)? When his clamp was kept tightened, the reaction could be repeated and "*though no inhibition occurred in the A-V junction, the block was again relieved.*" But as soon as pressure was released, vagal stimulation no longer relieved the block because, as he explains, the drug was then able to reach the compressed muscle to act upon it. The fact that the block is relieved after atropin injection, while the inhibitory action disappears, can be satisfactorily explained by the paralysis of the vagus fibers while the action of the sympathetic still goes through the compressed area, since it can be shown that other nerve fibers are less sensitive to pressure than the muscle

itself (Garrey, 1911; Meek and Leaper, 1912). Though the idea of the penetration of the drug to the compressed area is a very plausible explanation of disappearance of the reaction after releasing the clamp, it is important to have in mind that the action of this drug is not exclusively upon the parasympathetic (Danielopolu and Carniol, 1923) as the classical conception assumes, but that its pharmacological properties are more complex. Lewis, Drury and Bulger (1921), though aware that the drug determines a rise in the refractory period, conclude from their experience that it exerts no effect upon fiber conduction; but Petzetaki's experiments (1914, 1916) have conclusively proved that it exerts a clear negative dromotropic effect, the result of a direct action upon the intracardial conducting elements; and Meyer (1925), more recently, has pointed out that *in a second phase of action it paralyzes the sympathetic and thus exerts a negative dromotropic action.* This is so notable as to evoke in some instances an auriculo-auricular (see Petzetaki's and Meyer's tracings in papers quoted) and even sino-auricular block.

This paper does not pretend to resolve the problem, but after giving new evidence of the effects of sympathetic stimulation upon heart conduction, it demonstrates that there is but little evidence to support the idea that the vagus may exert the same effect, and strongly suggests on the basis of the same new and previous experimental evidence that such effects observed after vagus stimulation are to be considered as due to the stimulation of accompanying sympathetic fibers. A complete solution of the problem is urgent on account of its relations with the physiology of many pathological conditions and therapeutics of the heart.

SUMMARY AND CONCLUSIONS

As reasons for investigating the action of sympathetic cardiac nerves upon the turtle's heart-block, reference is made to conflicting and paradoxical effects upon heart conduction, attributed by different investigators both to sympathetic and parasympathetic sets of cardiac nerves.

The methods used for preparing the nerves for stimulation (see fig. 1) and registering the beats of the cardiac segments at both sides of the clamped areas are described. The care taken to avoid changes due to other factors than those tested in the experiment ("spontaneous" recovery; drying or remoistening effects; diffusion of current)—are emphasized.

Faradic stimulation of the fusiform ganglion (corresponding to the stellate ganglion of mammals) from which the more important sympathetic fibers issue to the heart, gave the following results:

a. *Intraventricular* block was never relieved.

b. *Auriculo-ventricular* block frequently was gradually relieved (see fig. 2), sometimes quickly (see fig. 4), and even abruptly reduced (see fig. 3). The effect may be so striking as to bring a severe degree of block (7:1) to a normal sequence of 1:1 (see fig. 4).

c. *Auriculo-auricular* block was relieved in conditions shown in figures 5 to 7, with a tendency to reappear after several minutes.

d. *Sino-auricular* block was quickly relieved.

Though in general a strong and often repeated faradic stimulus was necessary for obtaining these results, the nearer the blocked region to the sinus, the shorter the latent period required. This suggests that the turtle sympathetic exerts its action mainly upon the basal parts of the heart and probably upon the same side-half (see fig. 10).

All the features described fit well with those generally known of sympathetic stimulation.

Although improvement of conduction in any of the conditions studied was found not to be necessarily related to simultaneous accelerator or augmentor effects, they must be considered as characteristic sympathetic effects upon the heart.

These results are compared with those of vagus stimulation. Discussion of available anatomical and histological data and of previous work, and interpretations of other investigators and of experimental evidence presented in this paper are shown to indicate strongly that paradoxical improvement of conduction thus evoked is rather the result of stimulating sympathetic fibers going to the heart along the vagus nerve.

The disappearance of such vagal effects after atropin poisoning, cited as proof of their genuine vagal character, is questionable and only will be properly judged when we know better the complex action of this drug, especially upon the conducting system of the heart.

It is a pleasure to express my indebtedness to Dr. Walter E. Garrey for suggestions and criticism in carrying on this work. The author also wishes to thank the Staff of the Marine Biological Laboratory for the facilities received for carrying it along.

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THE INFLUENCE OF VARYING OXYGEN TENSIONS UPON THE RATE OF OXYGEN CONSUMPTION IN MARINE FISHES

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Received for publication December 17, 1928

Two views have been held in regard to the effect that variations in the oxygen supply have upon respiration exchange. The first is that the animal cell itself determines its own respiratory exchange and that the oxidative processes going on within it are independent, within wide limits, of the oxygen supplied. According to the second view, the variations in the oxygen supply must cause variations in the intensity of oxidations since the velocity of the reaction is held to depend upon the number of molecules able to take part in it. If the molecules of oxygen become fewer in number, the rate of oxygen consumption by the cells must decline unless their number is so large that it can be considered practically infinite compared with the number of molecules required to carry on normal respiration.

The first view is that of Pflüger (1873). It is somewhat speculative and teleological and assumes that the oxygen supplied to the cells is normally in excess of their requirements. Thunberg (Krogh, 1916) is the proponent of the second view, which is fundamentally opposed to that Pflüger, although he also assumes the oxygen supply to the tissues in the cases investigated by him was large enough to maintain a positive oxygen extension within the cells. Apparently he believes that catabolism of nutrient substances is a comparatively simple process of oxidation. The modern views of oxidation hold that such a process is very complex and must go through a number of rather definite intermediate stages. It is not yet known at what stages oxygen enters into the breakdown of nutrients or in how many stages it may play a part. Both Pflüger's and Thunberg's views lack sufficient evidence, and no generalization in this field is as yet satisfactory. There have been certain investigations, however, that shed light on some phases of the problem and offer some support of one view or another.

Since very little is known about the oxygen concentration within the cell either under normal conditions or under abnormally high or low oxygen tensions, all that can at present be done is to study the relation between the oxygen tension of the environment and the rate of oxidation within the

organism. It must be recognized that much of the information obtained in this way is unreliable. Investigation on single celled forms will perhaps in the end lead to the most satisfactory conclusions.

In the study of the influence of oxygen tension upon respiration, Amberson (1928) has shown that the oxygen consumption of Paramecia and Arbacia eggs is practically constant between 228 and 20 mm. Hg partial pressure of oxygen. Below 20 mm. Hg partial pressure the oxygen consumption is markedly reduced.

The studies on the influence of oxygen tension on the oxygen consumption of fish are meagre and few species have been used and these without regard to comparison with other species of different habits. A number of determinations were made by Winterstein (1908) on fresh water fish. These indicated that the oxygen consumption was practically independent of the oxygen tension down to about 2 per cent atmospheric pressure.

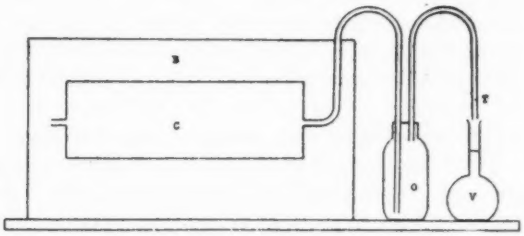

Fig. 1. Apparatus used for the determination of oxygen consumption of fishes at different oxygen tensions.

In the present investigations three species of marine fishes were chosen, the scup, *Stenotomus chrysops* (Linnaeus); the puffer, *Tetraodon maculatus* (Bloch and Schneider); and the toadfish, *Opsanus tau* (Linnaeus). The work was carried out in the U. S. Bureau of Fisheries laboratory at Woods Hole, Massachusetts. The author is indebted to the Bureau for assistance in the investigation, especially to Mr. Elmer Higgins for many courtesies in the laboratories, and Dr. I. E. Gray who aided in the analyses.

METHODS. The apparatus used in this investigation is shown in figure 1. It was a modification of the Ege and Krogh type of respirometer (Krogh, 1916) with a simpler means of determining the rate of flow of water and of taking the water sample. A large glass museum jar, *C*, of approximately 10 liters capacity was equipped at either end with a 6 mm. outlet tube and immersed in a large hatchery tank, *B*. One outlet tube connected the museum jar with a water sample bottle, *O*; the second tube, *T*, led to the sample bottle and was bent so as to allow all the water running through the museum jar to run into a volumetric flask, *V*.

Individual fish were placed in a small wire screen cage and supported in the center of the jar, *C*. Water was kept running constantly from a storage tank into tank *B* so that the overflow from it maintained a constant stream of water through the metabolism jar, *C*.

The rate of flow was determined by use of a stopwatch and a liter vol. flask. It could be varied by raising or lowering tube *T*, and in this manner the oxygen tension of the water surrounding the fish was controlled. The temperature was kept nearly constant throughout each experiment, between 20°–21°C. throughout the series of experiments.

In determining the oxygen consumption analyses were made of the dissolved oxygen in the water tank *B* and in the sample bottle *O* by the well-known Winkler method as modified by Birge and Juday (1911). Since organic matter might occur in sufficient concentration to interfere with the titrations, the samples were first treated with potassium permanganate and later decolorized by potassium oxalate. Knowing the oxygen concentration in cubic centimeter per liter in tank *B* and in bottle *O*, the rate of flow of water through the jar, *C*, and the weight of the fish, the oxygen consumption in cubic centimeter per kilo per hour was easily computed.

Fishes of nearly equal sizes were chosen in order to make the results comparable. They were also in good healthy condition. Scup were injected with urethane. Just a sufficient amount was given to prevent locomotor movements without apparently influencing respiratory movements. Such treatment was not necessary with either puffers or toadfishes since they both remained in a relatively quiet state throughout each experiment.

This method for the study of oxygen consumption of fishes has certain advantages over a closed container type from which samples are withdrawn at intervals. The water is kept in circulation, thus preventing stratification or uneven distribution of oxygen. Oxygen consumption from other sources than the fishes, such as bacteria and plankton, is practically eliminated. The oxygen samples may be taken more conveniently and with greater accuracy. The method is particularly adapted to the writer's experiments because the oxygen tension can be maintained constant at any level for any period of time by controlling the rate of flow of water past the fish. In the closed container method or in containers covered with oil, such as have been used in some investigations, the oxygen tension is not maintained at any one level but is lowered continually as the fishes remove oxygen from the water. Therefore results from such experiments do not accurately represent oxygen consumption at one oxygen tension level, but show the oxygen consumption at intervals when there is a continually decreasing oxygen pressure and are without regard to ajustment by the fishes to each level represented by analyses of the water samples. Employment of the method used by the writer, however, removes this

difficulty. During the experiments described in this paper several oxygen determinations were taken at intervals after an elapse of an hour which gave time for the adjustment of the fishes to the oxygen tension used. In this way fairly constant results were obtained and the determinations for several analyses were averaged in each case to express the influence of the oxygen tension upon the rate of oxygen consumption.

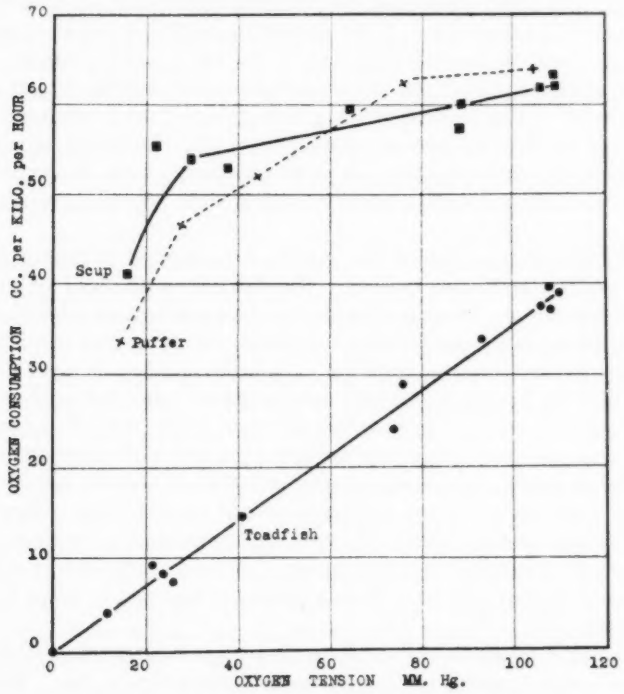

Fig. 2. Oxygen consumption of the scup, puffer and toadfish, at different oxygen tensions.

RESULTS. The results obtained in study of the influence of variations in the oxygen tensions on the oxygen consumption of the scup, puffer, and toadfish indicate that each species responds differently as indicated in figure 2. The scup shows very little change in its oxygen consumption with oxygen tension between 120 and 40 mm. Hg pressure. Below 30 mm. a decided drop in the oxygen consumption was obtained. The results were obtained with ten individuals. The oxygen consumption of the puffer apparently is influenced by any variation in the oxygen tension up to 100

mm. although the lower tensions cause the greater changes. The results were obtained with forty individuals. In the case of the toadfish the oxygen consumption was found to be directly proportional to variations in the oxygen tension between 0 and 118 mm. The results were obtained with eleven individuals.

DISCUSSION. It is apparent from the results obtained that oxygen tension has a differential effect upon the oxygen consumption of animals, even in species of the same class. It seems that the habits or degree of activity of a species may be correlated with the influence of oxygen tension. The scup is a relatively active fish and doubtless expends a great deal of energy during its life. The toadfish is sluggish and ofter lives in stagnant water and readily survives 24 hours in oxygen-free water. Its energy expenditure under fairly normal conditions is small compared with the scup. The puffer apparently occupies a position intermediate between the other two fishes.

In the case of the toadfish the oxygen consumption is almost directly proportional to the oxygen tension. This fish has no means of maintaining it at a constant rate. Perhaps this may be of some adaptive value inasmuch as the toadfish must many times encounter environmental conditions in which the oxygen becomes very low. Under such circumstances it would be benefited by having its oxygen consumption slowed below that of the normal rate. It is possible that this decrease in the rate of oxygen consumption may be only temporary, as such is the case in the earthworm (Lesser, 1908), in which after an anoxybiotic period there occurs a higher rate of oxygen consumption—a sort of oxygen debt is accumulated. This seems quite unlikely as the fishes were kept for hours under varying oxygen tensions with consistent results and little difference was observed in the influence of oxygen tension. More evidence is required in order to settle this matter.

Only a slight variation was shown in the oxygen consumption of the scup with the oxygen tensions ranging between 40 and 120 mm. Hg. Below 30 mm. Hg oxygen pressure, however, a very sharp decline in the oxygen consumption occurred and the fishes quickly died whenever the pressure fell below 16 mm. Hg. Toadfish lived for 24 hours with the oxygen tension between 0 and 1 mm. Hg pressure.

From some unpublished observations which the writer has made on the several species of marine fishes it appears that hemoglobin concentration may perhaps be an index of the physiological activity of a species. In other words, a fish having a higher concentration of hemoglobin is apparently able to expend its energy at a faster rate and conversely, a fish with low concentrations carries on metabolism at a lower rate. This was applied to the fishes studied in this paper with the following results:

	grams of hemoglobin per 100 cc. blood
Scup	8 3
Puffer	6 4
Toadfish	4 0

Thus the fishes having the higher hemoglobin concentrations, exemplified by the scup, are apparently less influenced by decreases in the oxygen tension down to about 30 mm. Hg partial pressure. The toadfish on the other hand has an extremely low hemoglobin concentration and has an oxygen consumption almost directly proportional to the oxygen tension.

The swimbladder may also be concerned in the influence that oxygen tension has on the rate of oxygen consumption by fishes. It has been shown that in some fishes this organ may act as a reservoir from which the blood draws oxygen when the oxygen tension of the surrounding water is low (Pearse and Actenberg, 1920; Hall, 1924). It seems improbable, however, that this would play a particularly significant part since the total quantity of oxygen contained within the swimbladder is relatively small (Hall, 1924) in comparison with the amount consumed during the time taken up by an experiment as performed in this investigation.

Krogh and Leitch (1919) investigating the dissociation of oxygen by the blood of fishes found that in fresh-water fishes, such as the carp, eel, and pike which are occasionally exposed to low oxygen pressures, the tension at which the blood unloads its oxygen in the absence of carbon dioxide is comparatively low, although higher than that for mammalian hemoglobin under similar conditions: but that it is greatly increased when there is an increase in carbon dioxide in the surrounding water. Marine fishes, cod and plaice, and the fresh-water trout, which are never exposed to low oxygen tensions, can unload oxygen from their blood, when it contains little or no carbon dioxide, at 15°C. nearly as well as mammals at 37°C. In these fishes also a small amount of carbon dioxide diminishes the affinity of the blood for oxygen. Krogh and Leitch consider the dissociation curve in both cases as specially adapted to the environmental conditions under which these fishes live.

There is conformity between the results obtained by Krogh and Leitch and those of the writer. The conclusion is apparently justified that there is correlation between the respiratory function of the blood of fishes and the systems of physiological activities occurring within their bodies. Such correlations to some degree furnish the basis for the adaptation of organisms to special conditions which may occur in their environment.

SUMMARY

1. The influence of oxygen tension on the oxygen consumption on three types of marine fishes was studied: the scup, a very active species, the puffer, a less active one, and the very sluggish toadfish.

2. There appears to be a correlation between the activity of fishes and the influence of the oxygen tension upon their oxygen consumption and the amount of hemoglobin per unit of volume in their blood.

3. The more active fishes show a greater resistance to variations in oxygen tensions than do sluggish fishes.

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THE GALVANIC SKIN-REFLEX AND FINGER VOLUME CHANGES

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Received for publication January 11, 1929

The fact that both electrical and vasomotor changes in the skin are known to follow bodily excitation has led numerous investigators to the inference that the electrical effects are of vasomotor origin.

The normal electrical effect of stimulation is a drop in resistance accompanied by potential changes in the skin areas to which electrodes are applied. The vasomotor effects, vasodilatation and vasoconstriction, are most frequently inferred from changes in the volume of parts of the body measured by plethysmographs. Calorimetric and microscopic methods of observation have been less commonly employed as indicators of the momentary effects following excitation. Vasoconstriction or reduction in volume of external parts of the body is the change most frequently and unambiguously measured by volumetric methods. A momentary increase in volume is often found to precede the more pronounced and obvious vasoconstrictor change following excitation. In some instances this may be due to a momentary increase in blood pressure.

The writers who have inferred that the electrical changes in the skin are of vasomotor origin may be divided into two classes: 1, those attributing the major electrical effect to vasodilatation, and 2, those who infer the cause to be vasoconstriction:

1. It appears to have been a natural inference that vasodilatation should effect a reduction in bodily resistance. Vigoroux (7) in 1879 suggested, on the basis of clinical evidence, that the diminution of resistance always depends on the dilatation and easy flow in the cutaneous capillaries. Féré (3) likewise in 1888 suggested that diminutions of the electrical resistance of the body are due to the increased irrigation of the tissues. Sticker (5) in 1897 suggested the possibility of the two electrical correlates, a negative change with vasodilatation, and a positive change with vasoconstriction. Later, in 1911, Radecki (4) attributed the galvanic reflex to changes in

[1] This is one of a series of studies from the Institute for Juvenile Research, Herman M. Adler, M.D., Director, Series B No. 134. The writer expresses appreciation of the assistance of Miss Lena Heath in the preparation of this material, and for the helpful criticism of the manuscript by Dr. Herman Adler, Dr. K. S. Lashley and Dr. A. J. Carlson.

CO_2 concentration which in turn he regarded as a function of vasodilatation and blood pressure.

2. Writers who have made attempts to obtain simultaneous records of vasomotor and electrical changes have been faced by the fact that not vasodilator (increased volume) but vasoconstrictor (decreased volume) changes are the observed effects, along with the galvanic skin-reflex, commonly following stimulation. Wiersma (9) in 1915 made simultaneous records of volume and electrical changes and noted that vasoconstrictor and electrical effects have similar clinical significance. Uhlenbruck (6) in 1924 obtained simultaneous records of volume and electrical changes from adjacent fingers of the same hand and showed that the galvanic and the vasoconstrictor changes are in most instances simultaneous in the subject on whom data are published. He explains instances when the two reactions appeared temporally separated or when one reaction occurred without the other as due to the possibility that different processes might be occurring in adjacent fingers. He infers that vasoconstriction and the galvanic reflex are but aspects of a general autonomic activity and that the studies in which one of these processes has been investigated may be interpreted in terms of the other measure. He avoids the inference that there is any causal relation between the two. Densham and Wells (2) and Wells (8) in 1927, however, reiterate the conclusion arrived at in 1925 by Aveling and MacDowall (1) that the "psychogalvanic" reflex is due to vasoconstriction and they declare the phenomenon to be the *"skin constrictor"* reflex. They believe that the skin is stretched by vasoconstriction to an extent sufficient to account for the reduction in resistance characteristic of the galvanic reaction. They base their opinion largely on the fact that both electrical and vasoconstrictor changes are likely to be elicited by the same stimulating physiological or pharmacological conditions and that both are likely to make a more or less contemporaneous recovery from excitation.

The purpose of this paper is to present evidence that the relation between vasoconstriction as indicated by volume change and the galvanic reflex as measured by the Wheatstone bridge method is casual rather than causal, and that such temporal relation as does appear is incidental rather than essential to the occurrence of either. A number of photographic records of simultaneous changes in finger volume, galvanic responses and blood pressure, obtained for another purpose, were analyzed to determine the temporal relation and the quantitative correlation of the electrical and plethysmographic changes. The record of blood pressure is of importance because of the apparent influence of internal hydrostatic pressure upon plethysmographically determined volume. Only volume changes in the finger attended by no variation or by an opposite trend of blood pressure may be considered unambiguous indications of vasomotor effects.

The apparatus is schematically represented in figure 1. The index and ring fingers of the left hand were enclosed in glass plethysmographs, *A*, *A*, fitted about the base of the fingers by collars made from lightly fitting finger cots. Care was taken that there should be no tightness, as any interference with venous return transforms a record obviously of vasomotor origin into one closely paralleling blood pressure. The hand was fastened by straps, *C*, *C*, to a board, *B*, which was suspended by cords from a support overhead. The finger plethysmographs, *A*, *A*, were connected by T and Y tubes with a flexible tube leading to a small delicate tambour acting on pointers throwing a shadow on the slit of a photokymograph. Zinc strips coiled within the

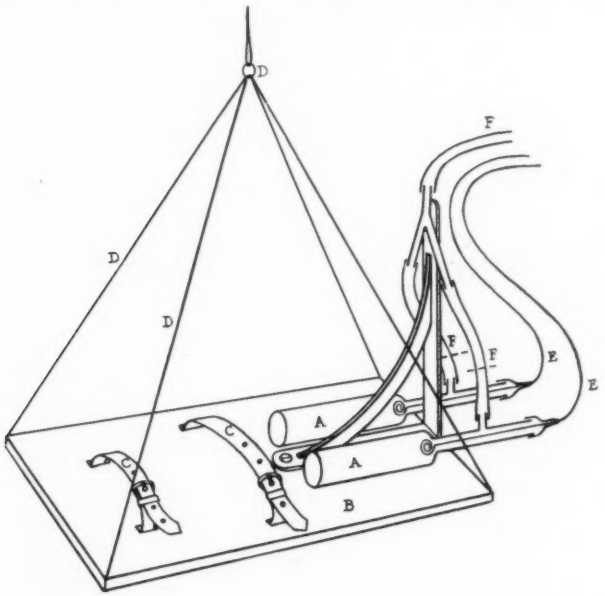

Fig. 1. *A*, *A*, finger plethysmographs; *B*, plethysmograph table; *C*, *C*, straps for hands; *D*, *D*, cords supporting table; *E*, *E*, leads to galvanometer; *F*, *F*, points of attaching clamps and approximate level of liquid during experimentation.

respective plethysmographs were connected by external wires, *E*, *E*, leading to a Wheatstone bridge, a 1½ volt dry cell, a suitable shunt and resistance, and a string galvanometer. When the plethysmographs were filled with dilute zinc sulphate solution rising approximately to the level of the dotted lines, *F*, *F*[2], they served as two liquid electrodes *rendering it possible to record changes in volume and in conductivity from identical areas of the body.*

The continuous record of blood pressure was obtained by means of a cuff on the arm opposite to that suspended by the swinging plethysmograph table. A pressure

[2] Clamps were applied at points *F*, *F*, and the cups containing liquid were held in a vertical position during adjustment to the subject.

of about 60 or 70 mm. Hg was maintained, sufficient to obtain clear-cut records of blood pressure changes and yet permit fair circulation. The arm was suspended in a sling at about the level of the head, reducing congestion in the hand and prolonging the period during which experimentation without discomfort could be continued. The blood pressure was recorded by a metal bellows acting by a rack and pinion upon a pivoted arm having two pointers separated by a constant angle. By using two pointers the one might be in a recording position after the other had been deflected from before the slit of the photokymograph. The duration of successive heart beats is carefully measured on the graphs by means of a pair of multiplying dividers, and the variations multiplied tenfold are projected in an inverted curve to show changes in pulse frequency.

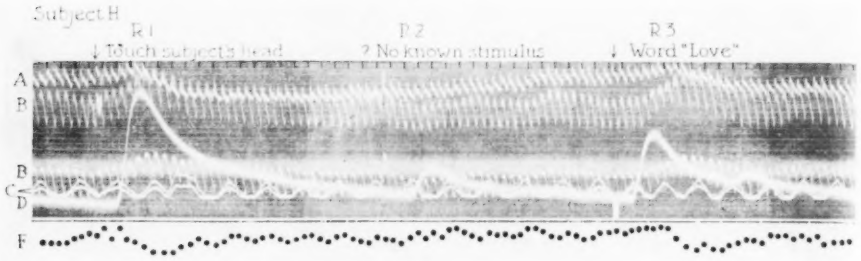

Fig. 2

Figs. 2, 3, 4, and 5. *A*, record of volume of ring and index fingers of left hand; *B*, *B*, continuous record of blood pressure from right arm obtained with inflation of 60 to 70 mm. Hg.; *C*, *C*, pneumographic records from chest and abdomen; *D*, record of changes in electrical resistance of the ring and index fingers, by the Wheatstone bridge method; *E*, vertical white lines in figures 3 and 5 indicate seconds. In figures 2 and 4, seconds are indicated approximately by black marks at the top of the record; *F*, pulse duration multiplied ten fold and shown in inverted curve as pulse frequency.

Sample records from different subjects taken by this apparatus, and graphs and tables representing data on all available material show:

1. The large degree to which blood pressure sometimes affects volume change (probably interpreted by some investigators as a vasomotor effect) in the fingers.

2. The widely varying temporal relation of vasomotor changes (volume changes which are not possibly attributable to blood pressure) to electrical effects.

3. The independent quantitative variation of the vasomotor and of electrical changes.

Figure 2 shows that the volume of the fingers may be influenced by blood pressure so as to parallel it closely:

R 1 and *3* show the galvanic skin-reflex occurring along with an increase in volume and blood pressure. Early vasoconstriction is here possibly obscured by increasing

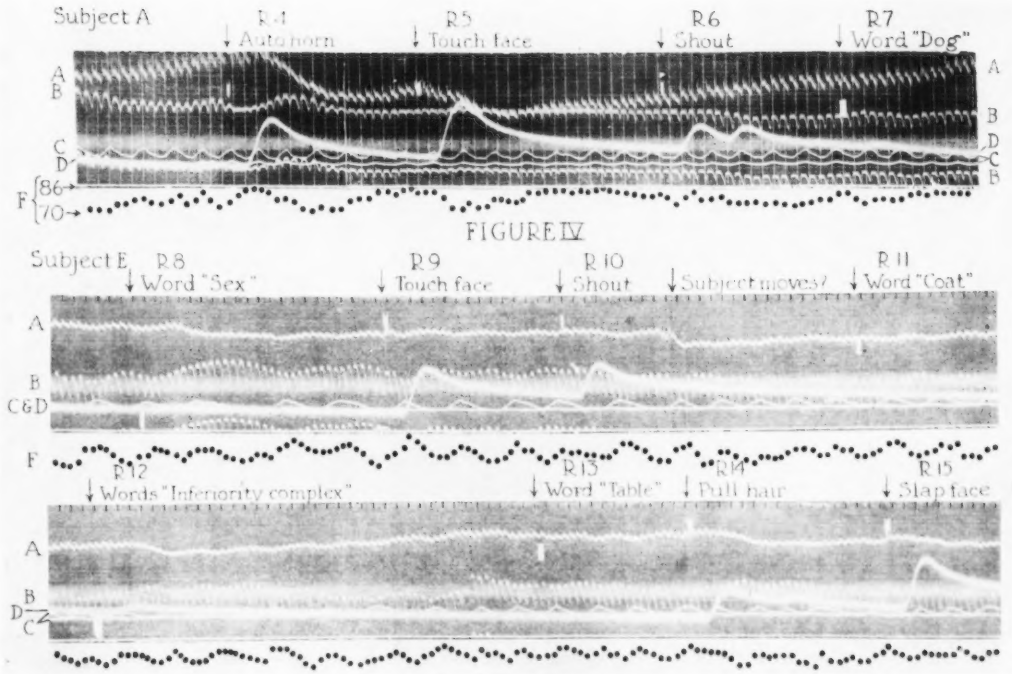

Fig. 3

FIGURE IV

Fig. 4

blood pressure. The fact that decreased heart rate follows the rise in blood pressure suggests that it is here a reflex compensatory reaction to increased pressure.

R *2* occurs during a slight drop in blood pressure. Vasoconstrictor changes, if any, are very small.

Figure 3 illustrates the independence of the galvanic skin-reflex, vasomotor and blood pressure changes:

R 4 shows the early phase of the galvanic skin-reflex attended by an increase in volume, little or no change in blood pressure, and an increased cardiac activity as indicated by pulse rate.

R 5 shows the galvanic skin-reflex occurring during a pronounced decrease in volume. A pronounced decrease in heart rate may here compensate for a tendency toward increased blood pressure due to vasoconstriction.

R 6 shows the reflex occurring twice without change in the trend of a continuous increase in volume which is opposite in direction to the trend in blood pressure.

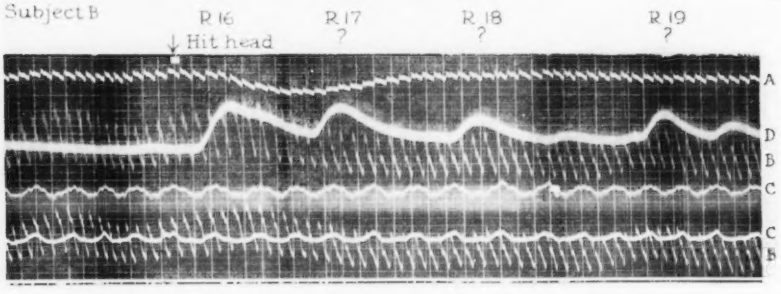

Fig. 5

Figure 4 shows from a continuous record of one subject the lack of correlation between the electrical and vasoconstrictor changes:

R 8 shows no galvanic reaction, although there is a marked vasoconstriction occurring in opposition to a rising blood pressure. Heart rate appears unaffected save by respiration.

R *9* and *10* show an electrical deflection unattended by any volume change which cannot be attributed to blood pressure.

R 11 shows only a rise in blood pressure following an ideational stimulus.

R 12 shows little galvanic reaction, although there is marked decrease in finger volume attended by a rising blood pressure.

R 13 shows no effect from an ideational stimulus.

R 14 shows a fair-sized galvanic reaction attended by circulatory changes similar in magnitude to R 12 which showed but slight electrical effect.

R 15 shows a larger galvanic reaction than R 14 but apparently with less vasoconstriction.

Figure 5 shows the fallacy of attributing the galvanic reflex to vasoconstriction on the basis of the fact that both are likely to follow the same stimulus:

R 16 shows an electrical change occurring during vasoconstriction.

R 17 shows the galvanic skin-reflex taking place during the refractory phase or recovery period of the vasoconstrictor mechanism.

R 18 shows an electrical change attended by little or no circulatory effect.

R 19 shows an electrical reaction occurring during no vasomotor change, or, if there is a change, during one that may be inferred from the blood pressure to be dilatation.

From these sample records of five different subjects, it is apparent that there is no necessary relation between the electrical and the volume changes

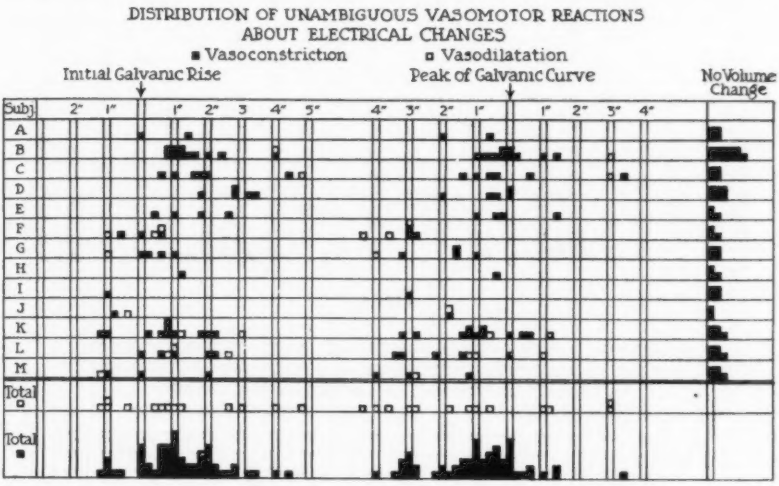

Fig. 6

Figs. 6, 7 and 8. The tables show instances of volume changes beginning during successive fifth-second periods before and after the galvanic changes. The times of the initial rise and of the peak of the galvanic curve are indicated by the respective arrows. Successive second periods before and after the electrical changes are numbered *1″*, *2″*, *3″*, etc. The same volume changes are presented in relation to the initial rise and to the peak of the galvanic curves.

in the fingers. An analysis of the temporal relations of the electrical to the volume changes of thirteen subjects confirms this observation. In figure 6 all volume changes which cannot possibly be attributed to blood pressure are presented in their temporal relation to the initial rise and to the peak of the galvanic curves. It is apparent that the beginning of vasoconstrictor changes is in the case of most subjects likely to follow the initial electrical deflection, and that all subjects display a widely varying interval between the onsets of the two changes. It further is evident that in most instances vasocontriction precedes by a widely varying interval the peak of

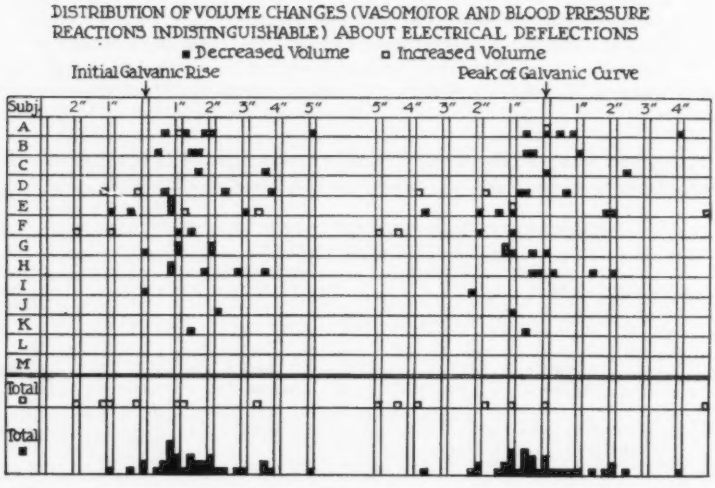
DISTRIBUTION OF VOLUME CHANGES (VASOMOTOR AND BLOOD PRESSURE REACTIONS INDISTINGUISHABLE) ABOUT ELECTRICAL DEFLECTIONS
■ Decreased Volume
□ Increased Volume
Initial Galvanic Rise
Peak of Galvanic Curve
Subj.
A
B
C
D
E
F
G
H
I
J
K
L
M
Total □
Total ■

Fig. 7

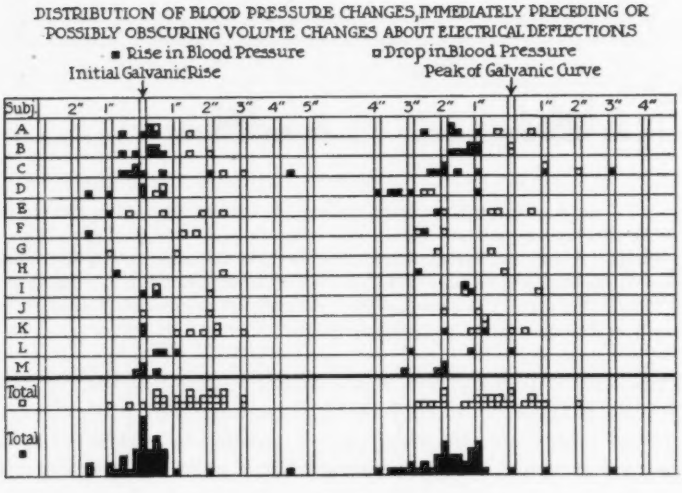
DISTRIBUTION OF BLOOD PRESSURE CHANGES, IMMEDIATELY PRECEDING OR POSSIBLY OBSCURING VOLUME CHANGES ABOUT ELECTRICAL DEFLECTIONS
■ Rise in Blood Pressure
□ Drop in Blood Pressure
Initial Galvanic Rise
Peak of Galvanic Curve
Subj.
A
B
C
D
E
F
G
H
I
J
K
L
M
Total □
Total ■

Fig. 8

the galvanic reflex curve. The number of instances in which electrical reactions were accompanied by no distinct vasomotor or blood pressure change is shown in the right hand column.

Figure 7 presents data from the same subjects, and similar to that in figure 6, save that in these instances blood pressure shows a change parallel to the plethysmographic changes, thus leaving no possibility that one will interfere with the other, but rendering the volume changes of ambiguous significance. Figure 8 gives instances of blood pressure change which immediately preceded an opposite vasomotor reaction, possibly delaying its appearance, or in some instances, possibly obscuring all such reaction. The

TABLE 1

Quantitative correlation, r, between the galvanic skin reflex and vasoconstrictor (decreased volume) changes during various phases of the electrical response

SUBJECT	N	CORRELATION OF TOTAL GALVANIC DEFLECTIONS WITH:			
		Volume change during latent period	Volume change during initial rise	Volume change during recovery period	Total of negative volume change*
A	22	+0.18	+0.57	+0.69	+0.73
B	38	+0.30	+0.43	+0.21	+0.42
C	24	+0.02	+0.03	+0.47	+0.51
D	27	−0.18	+0.24	+0.02	+0.04
E	26	−0.11	+0.32	+0.19	+0.24
F	34	−0.34	+0.45	−0.003	+0.23
G	12	−0.01	−0.11	+0.58	+0.55
H	26	+0.22	−0.07	−0.03	+0.09
I	16	+0.05	−0.36	+0.46	+0.41
J	24	+0.42	+0.04	−0.32	−0.02
K	25	−0.15	+0.49	+0.31	+0.40
L	13	+0.04	+0.25	−0.07	+0.13
M	14	+0.36	+0.47	−0.76	−0.51

A plus correlation indicates that the larger decreases in finger volume tended to accompany the larger galvanic changes.

* The sum of volume decreases during all three phases of the galvanic reflex.

striking feature of this graph is the close grouping of the first blood pressure changes about the moment of initial galvanic change. However, in only five of the instances of blood pressure rise beginning within one second before or after the initial galvanic change does pulse rate remain unchanged for the two or three seconds between the time when blood pressure begins to rise and the time when evidence of vasoconstriction appears. In the other cases a comparison of the rate during this interval with an equal interval just preceding shows heart rate to have increased in per minute rate by from 1.2 to 30.0 beats, with an average increase of 7.28, A. D. 3.97.

In other words, except in five instances, it does not appear necessary to assume vasoconstrictor effects which do not show on the records in order to account for the rise in blood pressure which frequently accompanies the initial galvanic deflection, and in these five instances of course there remains the possibility that they are due to an increased cardiac volume unaccompanied by a change in rate. But granting that in a fair proportion of the instances shown in figure 8 the rise in blood pressure was occasioned by a vasoconstriction which the blood pressure prevented from appearing as a volume change, this does not change the general inference to be drawn from the evidence. Similar instances of close temporal relation appeared in the data presented in figure 6, where occasionally both blood pressure and volume changes were in opposite directions and possibly counteracting one another, and in figure 7 where the changes were in the same direction with no apparent possibility of a mutually inhibiting effect.

As further evidence of the considerable degree of independence of these two reactions the correlations, r, between the total electrical change and volume changes during different temporal phases of the electrical reaction are presented in table 1. A perfect correlation of $+1.00$ would indicate that with every increase in the size of the galvanic change there was also an increase in the amount of vasconstriction. The possible effect of blood pressure upon the volume changes is not here taken into consideration. Without the elimination of this disturbing factor, it is apparent that there is little consistency of tendency toward correlation among different individuals for any of the three temporal phases of the galvanic change. Only total negative volume change tends in most instances to show some positive correlation with the galvanic skin-reflex. This is probably due to the fact that the two reactions are caused by the same stimulating conditions.

SUMMARY. The following evidence has been presented:

1. Evidence of an effect of blood pressure upon finger volume which must be taken into consideration when volume is used as an indication of vasomotor change.

2. Evidence that only infrequently is there an increase in finger volume unattended by a rise in blood pressure, thus rendering the evidence for dilatation in most cases ambiguous.

3. Evidence, in conformity with the findings of other investigators, that decreased volume (vasoconstriction?) is the typical response to stimulation.

4. Evidence of the widely varying temporal relation of the volume changes to the electrical variations in the same parts of the body (the fingers).

5. Evidence of the independent quantitative variation of volume and electrical changes in the same parts of the body.

CONCLUSIONS

From this evidence it may be concluded:

1. That in so far as volume changes may be accepted as indicators of vasomotor phenomena there is little consistent evidence of vasodilator changes (increased finger volume not attributable to blood pressure) following stimulation.

2. That, in so far as volume changes may be accepted as indicators of vasomotor phenomena, there is evident no necessary relation between vasoconstriction and the galvanic skin-reflex.

3. That vasoconstrictor and electrical changes are related phenomena in the sense that they are functions of common stimulating conditions.

Note: That there are vasomotor phenomena not made evident by records of volume change, and, perhaps, related to the electrical changes, will be shown in a succeeding paper.

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THE PERMEABILITY OF THE PLACENTA OF THE RAT TO GLYCINE, ALANINE AND UREA

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Received for publication December 12, 1928

In most of the studies on placental function in which attempts have been made to reveal the mechanism by which metabolites are conveyed to and from the fetus, it has been customary to base the conclusions upon simultaneous analyses of maternal and fetal blood at term. In general, if the concentration of a given metabolite in the maternal plasma proved to be equal to its concentration in the fetal plasma, the placenta was considered to be freely permeable to that metabolite. By this means it has been demonstrated that creatine and creatinine (Hunter and Campbell, 1918), magnesium (Bogert and Plass, 1923), urea (Plass and Matthew, 1925), and uric acid (Slemons and Bogert, 1917, and Plass and Matthew, 1925) pass by diffusion through the placenta.

On the other hand, any inequality in the concentrations of the metabolite in the two fluids has been taken to indicate placental activity of a more complex kind. Thus it has been contended that the higher level of amino nitrogen (Hellmuth, 1924, Plass and Matthew, 1925, Morse, 1917, and Rabinovitch, 1914), calcium (Bogert and Plass, 1923, Bokelman and Bock, 1928, and Hellmuth, 1925), and inorganic phosphate (Oettingen, 1925, and Plass and Tompkins, 1923) in the fetal blood is evidence of secretory activity on the part of the placenta operating to the advantage of the fetus.

To weigh properly the merits of this hypothesis it is necessary that more be known of the fate of a metabolite which has suffered a great increase in concentration in the maternal plasma. Does it pass readily though the placenta or is it barred completely or in part? Is there an increase of similar magnitude in its concentration in the fetal blood plasma, is it rapidly catabolized in the fetus, or is it fully retained by—the maternal organism?

Because of the technique generally adopted, viz., analysis of maternal and fetal blood at the time of delivery, this necessary information has seldom been obtained. It is urgently needed for a number of disputed cases, especially for the amino acids, whose normal distribution between

maternal and fetal blood at term is sufficiently uncertain to envelop the whole problem in obscurity. The equality in distribution observed by Morel and Mouriquand (1913) and inferred from non-protein and urea determinations by Slemons and Morriss (1916) and Caldwell and Lyle (1921), would suggest a ready diffusion of the amino acids through the placenta. On the contrary the observations of Bock, Hellmuth (1924), Morse (1917) and Plass and Matthew (1925) which demonstrated the fetal plasma values to be somewhat greater than those of the maternal plasma have suggested to some the existence of a diffusion gradient from the fetus to the mother or of secretory activity on the part of the placenta.

Although we have not found it feasible to investigate the problem by plasma analyses, we have attempted to supplement the knowledge of placental permeability by analyses of the whole fetus and maternal liver and muscle. By so doing we have been able to increase at will the concentrations of the selected metabolites in the maternal organism and to determine the changes if any in the concentrations within the fetus. By examination of animals in series at different times after injection of the experimental substances it has been possible, moreover, to study the time relationships involved and hence to ascertain the rates of placental response.

EXPERIMENTAL. Twenty-four rats of standard stock taken on the eighteenth or nineteenth day of gestation were used. At this time the average fetal weight was 1.1 to 3.0 grams, the weights for a given age being dependent largely upon the number of the fetuses in the uterus.

Glycine, alanine, and urea were administered as the experimental substances, saline being administered to the controls. The two former were selected in preference to other amino acids since their absorption is attended by particularly high amino nitrogen values in the blood, because much is known of their post-absorptive transfer in the adult rat (Luck, 1928, and Luck and Specker) and because of their great solubility. Under the conditions adopted in this work, the amino nitrogen content of the maternal blood would be increased two- to threefold as a consequence of the administration of glycine and alanine, and would be maintained at this high level for two hours or more (Luck and Specker). This result is of considerable value when placental permeability is the object of study.

After a fasting period of 20 hours, the animal received the metabolite by subcutaneous injection. The material was dissolved in 5 cc. of 0.9 per cent sodium chloride, the quantity chosen for administration being the equivalent of 0.4 gram nitrogen per kilo body weight. From 15 minutes to 7 hours after injection the animal was lightly anesthetized with ether. The uterus was rapidly excised, several of the fetuses, enough to give about 6 grams of tissue, were removed, quickly sponged off on filter paper, and dropped into liquid air. The liver and the thigh muscles of one limb of the pregnant animal were likewise removed and promptly

frozen. The tissue samples thus obtained were powdered, and weighed portions analyzed for urea and amino acid nitrogen according to the methods of Luck (1928), and Kiech and Luck (1928), respectively. Nine animals were employed in the experiments with glycine, six with alanine, and four with urea. Five animals which received injections of saline only, served as controls.

The amino nitrogen values for the glycine series are presented in figure 1, and for the alanine, in figure 2. It should be observed that the graphs are composite, all points on any given curve representing separate analyses of as many different animals. Thus from the 9 animals employed in the glycine series, the nine points on the liver curve, the nine on the muscle curve and the nine on the fetal curve were obtained. In figure 3 are presented in similar fashion the urea values obtained after administration of urea.

Discussion. On examination of figures 1 and 2 a very close similarity is revealed in the responses of maternal muscle and the fetus to the inflowing amino acids. The muscle-glycine and fetal-glycine curves are identical in form, as are also the muscle-alanine and fetal-alanine curves. The parallelism of the muscle and fetal amino nitrogen curves is rendered more apparent by contrast with the liver amino nitrogen curves. The latter reveal the interesting fact that amino acids, though subcutaneously administered, are absorbed with astonishing rapidity by the liver. The great increase in the amino nitrogen content of that organ leads one to suspect that the liver possesses a marked affinity for the amino acids and selectively absorbs them from the blood. The similarity in the fetal and the maternal muscle amino nitrogen curves suggests that the absorption of amino acids by the maternal muscle is by essentially the same mechanism as that by which the amino acids pass from the fetal blood to the fetal tissues and that this mechanism in turn is quite different than that which operates in amino acid absorption by the liver. Maternal plasma contains normally 7 to 10 mgm. of amino acid nitrogen per 100 cc., while maternal muscle has a normal content of 50 to 55 mgm. of amino acid nitrogen per 100 grams. By whatever means this inequality in distribution is to be explained, it is worth observing that a similar inequality exists in the fetus. The entire fetus contains 44 to 49 mgm. of amino acid nitrogen which is essentially the same as that of the normal adult (Kiech and Luck, 1928) while the blood plasma of the new-born probably contains only 1 to 3 mgm. more amino acid nitrogen per 100 cc. than that of the mother, viz., 9 to 12 mgm.

In addition to any conclusions, such as the above, which are based upon the identity in form of the muscle and fetus curves it is possible to draw an inference with respect to the permeability of the placenta. Following the administration of glycine, there is a rapid though small increase in the

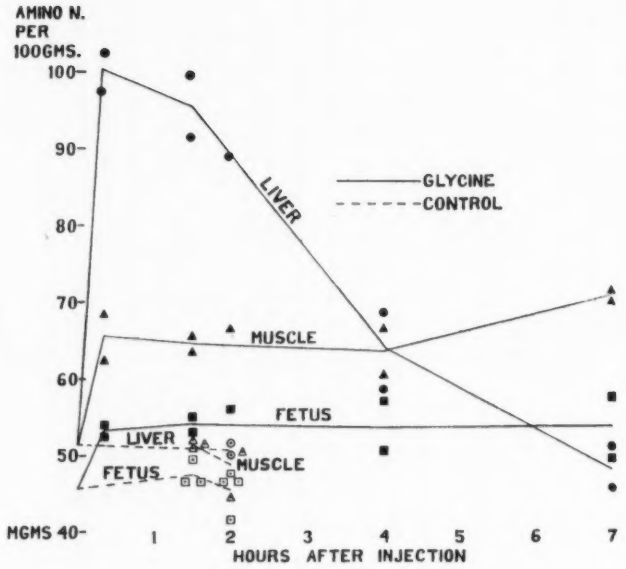

Fig. 1

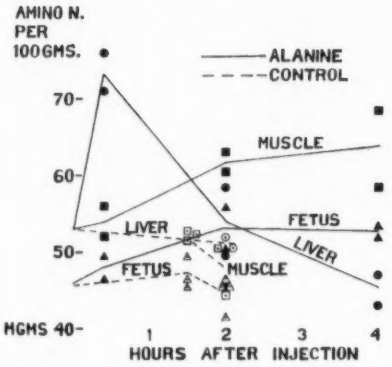

Fig. 2

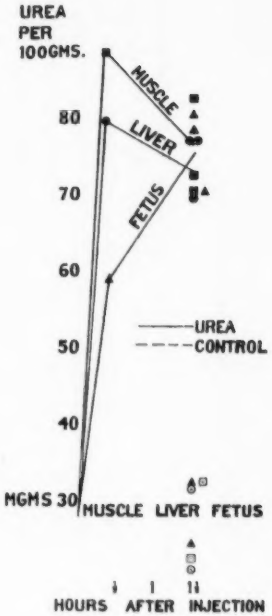

Fig. 3

Fig. 1. Analysis of maternal liver and muscle and the whole fetus after injection of glycine
Fig. 2. Analysis of maternal liver and muscle and the whole fetus after injection of alanine
Fig. 3. Analysis of maternal liver and muscle and the whole fetus after injection of urea

amino nitrogen content of the maternal muscle and fetus, an approximately steady state being rapidly attained. In this condition the amino nitrogen concentration undergoes but little change. The same is true of the alanine experiments except that the absolute increases are less and the steady state is more slowly attained. If we now compare the absolute increases in the amino nitrogen values of maternal muscle and the fetus after the steady state has been reached, we find that the administration of glycine caused an increase of about 8 mgm. in the fetal amino nitrogen, and of 13 mgm. in the muscle amino nitrogen. The corresponding increases after administering alanine were about 7 mgm. and 10 mgm. respectively. Bearing in mind the apparent similarity in the mechanisms by which the fetus absorbs amino acids from the fetal plasma and the maternal muscle absorbs amino acids from the maternal plasma, one feels compelled to conclude that the amino nitrogen increases in the two plasmas must have been of much the same magnitude. If the increase in the amino nitrogen content of the maternal blood was two- to threefold (Luck and Specker) that of the fetal blood cannot have been very much less. In consequence it appears most likely that the passage of amino acids through the placenta is primarily one of ordinary diffusion.[1] This conclusion is supported by the observations of Piccoli (1920) which indicate that if the amino nitrogen of the maternal blood be artificially increased, that of the fetal blood suffers a similar increase. Likewise, from the data of Morse's experiments (1917) it may be observed that in two cases of hyperaminoacidemia in which the amino nitrogen values for the maternal bloods were 21.6 and 21.4 mgm., those of the fetal bloods were 21.4 and 24.4, respectively.

Indeed the only reason presented by investigators in support of a mechanism more recondite than that of diffusion appears to be the fact that the amino nitrogen content of the fetal plasma at birth is somewhat higher (about 2 mgm.) than that of the maternal plasma. Since the placental barrier may be considered as analogous to a semi-permeable membrane there can be little doubt that the distribution of electrolytes on either side will partly be of the type studied by Donnan. If so, it should be possible to explain inequalities in the concentrations of readily diffusible

[1] We gave some consideration to the possibility that protein catabolism in the fetus might proceed with greater velocity than in the maternal tissues. If so deamination of the amino acids absorbed by the fetus would proceed more rapidly than in the maternal tissues. The tendency would be to establish a lower amino acid level in the fetus which might indeed serve to explain the fact that the increases observed by us in the amino nitrogen of the fetus were somewhat smaller than those of the maternal muscle. If this were an acceptable explanation the urea content of the fetus at the height of amino acid absorption should tend to be higher than the urea content of the maternal tissues. On the contrary we found the fetal urea values to be equal to those of the maternal tissues or, if any difference existed, to be actually lower in the fetus.

electrolytes in maternal and fetal plasma without postulating the secretion of amino acids by the placenta. It is probable, moreover, that the normal distribution of amino acids on either side of the placenta would be greatly influenced by the hydrogen ion concentration in maternal and fetal blood. Mendéléeff (1923) has reported that the serum of the fetal guinea pig at term is much more acid (pH = 6.2) than the maternal serum. If this is true the amino acids of the former may exist more largely in the undissociated form. This would influence the solubility of the amino acids, their diffusion velocities and the magnitude of the Donnan effect exerted by them.

Finally, from our knowledge of the passage of many other substances through the placenta we have good cause to suspect that it would be readily permeable to substances of small molecular size and great diffusion velocity, like the amino acids. For it is apparently established that the placentae of guinea pigs, rabbits and humans are uniformly permeable to such large particles as the protein-sensitizing bodies, antitoxins, agglutinins, precipitins, and bacteriolysins. The placentas of the cow, sheep, and horse belong to a different type and are uniformly impermeable to these bodies. The extensive literature dealing with these problems has been reviewed by Ratner, Jackson and Gruehl (1927).

The experiments with urea are more easily interpreted. For unlike our investigation with the amino acids there are no complicating factors such as catabolism of the absorbed substance, and great inequality in its normal distribution between plasma and tissues. Within two hours of administration of the material the urea concentrations of muscle, liver, and fetus had become equal though much higher than in the normal state (fig. 3). There can be no doubt that urea diffuses readily, not only through the placenta but into other maternal and fetal organs.

SUMMARY

1. Glycine, alanine, and urea in equimolecular amounts were administered by subcutaneous injection to pregnant rats on the 18th or 19th day of gestation. At varying intervals after injection the animals were anesthetized and the fetus and maternal liver and muscle were analyzed for amino acid nitrogen and urea.

2. After administering glycine and alanine the rates of increase in the amino nitrogen content of the maternal muscle and the whole fetus were very similar. The identity in form of the muscle and fetus amino nitrogen curves and the size of increase in the amino nitrogen values above the basal, indicate that the placenta is quite permeable to amino acids.

3. The mechanism by which the maternal muscle absorbs amino acids from the maternal plasma is apparently the same as that by which the fetus absorbs amino acids from the fetal plasma.

4. Amino acids are absorbed from the systemic blood by the liver with very great rapidity. The mechanism appears to be quite different than that which operates in muscle.

5. Urea diffuses readily through the placenta.

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PROOF THAT THE STIMULATING EFFECT OF FAT ON METABOLISM IS DUE TO THE FATTY ACID RADICAL OF THE FAT MOLECULE AND NOT TO THE GLYCEROL RADICAL

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Received for publication December 17, 1928

Lavoisier (1780) showed that the ingestion of food increased oxidation in the body. Rubner (1902) found that fat increased oxidation or metabolism more than did sugar and less than protein. The object of this investigation was to determine whether the fatty acid radical or the glycerol radical of the fat molecule or both are responsible for the stimulating effect of fat on metabolism or, more specifically, sugar metabolism. The respiratory quotient is the index usually used for the amount of sugar metabolized, a rise in the quotient indicating an increase in sugar metabolism, and a fall a decrease. In this investigation sugar utilization, as well as the effect of the fatty acids and glycerol on its utilization, was determined directly according to the following procedure.

Nine hundred cubic centimeters of 0.1 per cent dextrose solution were prepared and divided into portions of 100 cc. each. These 100 cc. portions were introduced into beakers. Ten milligrams of sodium oleate were introduced into one beaker; 10 mgm. of potassium oleate into a second; 5 mgm. of sodium palmitate into a third; 10 mgm. of potassium palmitate into a fourth; 15 mgm. of sodium stearate into a fifth; 15 mgm. of potassium stearate into a sixth, and 200 mgm. of glycerol were introduced into a seventh. Two gold fish of approximately the same size and with a combined weight of approximately 5 grams were then introduced into each of the beakers. Air was bubbled through the sugar solutions to insure an adequate supply of oxygen to the fish. The two beakers containing gold fish and sugar solutions to which nothing was added served for controls. Sugar determinations were made according to the method of Benedict immediately and after 30 hours. The average for 5 series of experiments are shown in figure 1. It will be seen that the average amount of sugar used in 30 hours by the controls was 30 per cent; that the fish in the sugar-sodium oleate solution used 47 per cent; the fish in the sugar-potassium oleate solution, 52 per cent; those in the sugar-sodium palmitate solution, 51 per cent; those in the sugar-potassium palmitate solution, 49 per cent; those in the sugar-sodium stearate solution, 56 per cent; those in the sugar-

potassium stearate solution, 42 per cent and those in the sugar-glycerol solution, 32 per cent. By comparing these figures it will be seen that the fatty acids, or rather the sodium and potassium salts or soaps, produced a marked increase in sugar utilization, while the glycerol had practically no effect.

The question might be raised whether it was the sodium or potassium or the acid radical in the fat molecule that was responsible for the stimulating effect on sugar metabolism. Sodium chloride and potassium chloride were tried and they were found to have no effect on sugar metabolism. Hence it may be concluded that neither the sodium nor the potassium radi-

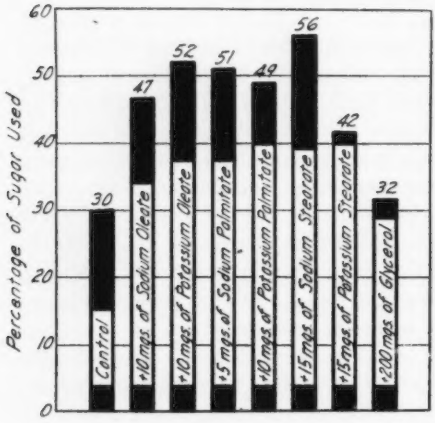

Fig. 1

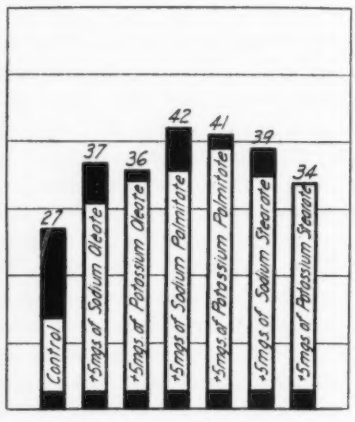

Fig. 2

Fig. 1. Chart showing that the sodium and potassium salts of the fatty acids, oleic, palmitic and stearic increase sugar metabolism while glycerol does not.

Fig. 2. Chart showing that of the three fatty acids oleic, palmitic and stearic probably palmitic has the strongest stimulating effect on sugar metabolism.

cal had any effect, so the fatty acid radical must have been responsible for the stimulation. It should also be stated that the effect of inorganic salts, such as the phosphates, carbonates and sulphates of potassium, sodium, calcium and magnesium were also studied and it was found that only the phosphates stimulated sugar metabolism.

The amount of the fatty acids used was determined by their toxicity. The maximum amount that would not kill the fish in 30 hours was used in every case. Sodium palmitate was found to be the most toxic and only 5 mgm. of this substance could be used without killing the fish. Although much more glycerol was used than could possibly have been derived from the digestion of fats represented by the amounts of the different fatty

acids used, it had practically no effect on sugar utilization. A very large quantity of glycerol, 500 mgm., was found to increase sugar metabolism but slightly. It was found, however, that other alcohols, methyl and ethyl, produced a marked stimulating effect on sugar metabolism. The effect of the higher alcohols, amyl, propyl, and butyl, was also studied. These alcohols, however, were found to be so toxic that the work with them was discontinued.

The following experiments were performed to compare the stimulating effect on sugar metabolism of the different fatty acids used in the previous experiments. For this purpose a similar amount of each, viz., 5 mgm., was added to 100 cc. of 0.1 per cent dextrose solutions in beakers in each of which were placed two gold fish as was done in the preceding experiments. Sugar determinations were made immediately and at the end of 30 hours. The results of the average of five series of experiments are shown in figure 2. By comparing the percentage increase in sugar utilization, when equal amounts of the different salts were used, it will be seen that the palmitates produced a slightly greater increase in sugar metabolism than the oleates or stearates.

The following precautions were taken and checks made in the preceding experiments. Air was bubbled through sugar solutions for 30 hours without the presence of fish and it was found that this had practically no effect on the sugar solutions. Air was also bubbled for 30 hours through sugar solutions into which had been introduced the different sodium and potassium salts of the fatty acids used in the experiments without any effect on the amounts of sugar in the solutions. It was found that the sugar was used only when the fish were present and the utilization ceased upon the removal of the fish. From these observations it was concluded that the fish were responsible for the utilization of the sugar observed in these experiments and not bacteria or yeast. Distilled water and not tap water was used in preparing the solutions to prevent the precipitation of the soaps by salts, such as those of calcium, in the tap water.

SUMMARY

1. The ordinary fatty acids, oleic, palmitic, and stearic stimulate sugar metabolism while glycerol does not.

2. Palmitic acid is more toxic and stimulates sugar metabolism slightly more than oleic or stearic.

3. The conclusion is drawn that the fatty acid radical in the fat molecule and not the glycerol radical is responsible for the stimulating effect of fat on sugar metabolism.

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THE EFFECT OF FASTING UPON CERTAIN PHASES OF CARBOHYDRATE METABOLISM

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Received for publication December 28, 1928

It has been known for some time that alterations in the diet may modify carbohydrate tolerance. Most of the theories advanced to explain these changes emphasize the possibility of the effect being due to alterations in the internal secretion of the pancreas. The possibility that other factors may play an important rôle has not been ignored. The literature concerning directly or indirectly this phase of carbohydrate metabolism is entirely too voluminous to permit more than an occasional reference to the observations of many investigators. In a previous communication the authors (Boyd, Hines and Leese, 1925) called attention to the marked uniformity in the response of a given animal to repeated injections of glucose when the animal was observed under similar physiological conditions. This was later confirmed by Jordan (1927). This communication deals with the effect of fasting upon the response of dogs to glucose administration by the continuous intravenous method at the rate of 4 grams per kilo per hour for a period of 2 hours. Observations were made before, during and after the period of glucose administration on blood sugar, urinary glucose, plasma hemolgobin, pH and CO_2 content, serum inorganic phosphate, respiratory quotient and heat production. The experimental methods employed were essentially the same as those previously described (Boyd, Hines and Leese, 1925). A control period was run on each animal before and again after ample time had been allowed for recovery from previous fasts. All control periods were run 20 hours after food ingestion. Extension of time beyond this is considered to be the duration of the fasting period. Nine experiments were made on four animals at the termination of the fasting period. The length of the fasts varied from 48 to 144 hours. Water was allowed ad libitum in all periods.

The data presented in this paper are in harmony with the findings of Bang (1913), Staub (1922), du Vigneaud and Karr (1925), Traugott (1922) and other investigators in that fasting decreases carbohydrate tolerance. This was evidenced by the finding of a greater degree of hyperglycemia (table 1) and glycosuria (table 2) in experiments where glucose injec-

TABLE 1

Blood sugar changes

AVERAGES	BEFORE INJECTION	END OF ½ HOUR OF INJECTION	END OF 1 HOUR	END OF 2 HOURS	END OF ½ HOUR POST INJECTION	END OF 1 HOUR POST INJECTION
7 control periods on 4 animals	0.101	0.283	0.492	0.400	0.187	0.079
4 fast periods of 48 hours on 3 animals	0.090	0.341	0.640	0.585	0.259	0.115
4 fast periods of 72–96 hours on 4 animals	0.082	0.391	0.726	0.761	0.395	0.213

TABLE 2

Per cent excretion of injected glucose and changes in composition of blood caused by glucose injections

CONDITION OF ANIMAL	PER CENT GLUCOSE EXCRETED	PLASMA pH			PLASMA CO_2			SERUM† PHOSPHATE			PER CENT Hb* END OF INJECTION
		Before	End	Difference	Before	End	Difference	Before	End	Difference	
700 A control	23.4				44.4	40.6	−3.8	4.7	2.6	−2.1	97
700 B 72 hour fast	42.5				50.7	34.8	−15.9				99
700 C 48 hour fast	21.1	7.35	7.28	−0.07	41.0	33.6	−7.4	5.9	2.1	−3.8	89
700 D 48 hour fast	21.0										
700 E control	19.4	7.35	7.40	+0.05							94
701 A control	22.4	7.41	7.39	−0.02	51.4	45.0	−6.4	3.7	2.5	−1.2	96
701 B 72 hour fast	42.6				49.8	38.8	−11.0	3.6	3.1	−0.5	90
701 D 144 hour fast	35.0	7.35	7.28	−0.07	49.1	43.5	−5.6	4.1	3.3	−0.8	80
702 A control	11.7	7.44	7.36	−0.08	46.9	41.5	−5.4	5.0	3.1	−1.9	91
702 C 48 hour fast	15.3	7.37	7.35	−0.02	43.9	31.4	−12.5	5.0	3.6	−1.4	95
702 D 96 hour fast	18.2	7.33	7.28	−0.05	46.4	36.2	−10.2				86
702 E control	14.7	7.36	7.34	−0.02	46.3	40.4	−5.9	3.1	1.4	−1.7	72
703 A control	19.1	7.39	7.37	−0.02	51.4	45.8	−5.6				91
703 B 48 hour fast	24.0	7.40	7.37	−0.03	55.2	41.3	−13.9	4.0	1.8	−2.2	91
703 C 96 hour fast	26.0	7.39	7.26	−1.3	52.8	35.5	−17.3	5.9	3.7	−2.2	
703 D control	7.5	7.40	7.39	−0.01	50.3	45.4	−4.9	4.5	2.1	−2.4	80
Average controls	16.9	7.392	7.375	−0.017	48.5	43.1	−5.4	4.2	2.3	−1.9	89
Average fasting	27.3	7.365	7.303	−0.062	48.6	36.9	−11.7	4.8	2.9	−1.9	90

* Initial values equal 100 per cent.

† Mgm. P_2O_5 per 100 cc.

tions were made during the fasting periods rather than during the control experiments on the same animals. In general, the degree of lessened capacity to handle glucose varied with the length of the fasting period. However, the amount of the injected glucose retained by the 48 hour fasting animals fell within the limits of the controls in three out of four experiments. The effect upon the degree of hyperglycemia was more pronounced than upon the amount of glycosuria. In the longer fasting periods, the percentage of injected glucose eliminated was approximately twice that in the control periods. The respiratory data (table 3) indicate that fasting has been without appreciable effect on the quantity or quality of the oxidative processes occurring during the time of glucose administration. The

TABLE 3

Respiratory quotient and calories per square meter of body surface per hour

Based upon Rubner's constant in Meeh's formula $\sqrt[3]{11.2 \text{ weight in grams}^2}$

ANIMAL NUMBER	NATURE OF EXPERIMENTS	PRELIMINARY		INJECTION PERIOD*		POST INJECTION*	
		R. Q.	Calories	R. Q.	Calories	R. Q.	Calories
700 avg.	Control	0.79	39.90	0.98	55.43	0.86	45.65
700 avg.	Fasting	0.72	37.64	1.08	56.86	0.85	35.60
701 avg.	Control	0.79	35.49	0.99	52.14	0.85	44.53
701 avg.	Fasting	0.70	39.13	0.99	52.38	0.83	36.32
702 avg.	Control	0.80	41.88	0.99	59.84	0.84	43.59
702 avg.	Fasting	0.67	35.34	0.92	63.25	0.84	33.86
703 avg.	Control	0.79	40.18	0.95	61.64	0.84	37.99
703 avg.	Fasting	0.69	40.18	0.97	57.62	0.84	42.54
Avg.	Control	0.79	39.36	0.98	57.26	0.85	42.94
Avg.	Fasting	0.70	38.07	0.99	57.53	0.84	37.08

* Averages of 2 or more determinations made in each period.

R. Q. quickly approximated unity and remained at this value throughout the injection period. Glucose administration was accompanied by an increase in heat production of approximately 50 per cent in both control and fasting periods. Hence the quantity of glucose disposed of by oxidation was essentially the same in both series.

It is evident from the data presented in table 2 that as far as can be ascertained from studies of plasma pH and CO_2 content, these periods of fasting were not accompanied by any significant changes in the acid base balance. The somewhat greater fall in plasma pH and CO_2 content found to have occurred at the end of the period of glucose injection in the fasting animals is regarded as being without significance. We have frequently found as great a decrease as this in experiments on non-fasting animals exhibiting similar degrees of glycosuria and hyperglycemia. No difference was found

in the extent of the fall in serum inorganic phosphate during the course of glucose injection. The extent of the blood dilution as judged by hemoglobin changes was the same in the fasting and control experiments.

In order to properly correlate our findings with those of previous investigators one must keep in mind the variance in the experimental methods employed. Fasting in the dog is not accompanied by the degree of ketosis and acid base changes found in some other experimental animals and in man. The studies made in these experiments indicate that no appreciable change in the acid base balance of the blood had occurred at the time of testing the efficiency of the carbohydrate handling mechanism in the fasting periods. The blood sugar changes were qualitatively the same in the control and fasting experiments. The greater degree of hyperglycemia during the injection and the delayed return of the blood sugar to preliminary values in the post injection period noted in the experiments on fasting animals might be considered as evidence for a lack of available pancreatic hormone. However the finding of the same degree of fall of inorganic phosphate in the blood in the control and fasting experiments is not favorable to this assumption. Further, the finding of the same degree of increase in the R. Q. and heat production during glucose administration in the fasting and non-fasting experiments does not reflect an inadequate supply of pancreatic hormone. The latter finding is not in harmony with the observation of Goldblatt (1925) who noted that glucose administration failed to cause the usual increase in R. Q. during a fast in man. This he interpreted as indicating a lack of pancreatic hormone.

The data considered collectively indicate that the difference between the response of a fasting and a non-fasting animal to glucose injections is a quantitative one and not a qualitative one. It is known that fasting may diminish the degree of functional activity of many other physiological processes. It is suggested that the lowered carbohydrate tolerance in fasting should be regarded as an impaired function of the organism as a whole rather than a specific effect upon any one mechanism.

SUMMARY

Comparisons were made of the response of non-fasting and fasting dogs to a continuous intravenous injection of glucose at the rate of 4 grams per kilogram of body weight per hour for a period of two hours. Animals in the fasting condition exhibited a greater degree of glycosuria and hyperglycemia than the non-fasting controls. The extent of the diminished tolerance varied, in general, with the length of the fast. Animals in the fasting and non-fasting condition exhibited the same increase of R. Q. and heat production, fall in serum inorganic phosphates and blood dilution. No significant changes in the acid base balance of the blood was noted.

The difference in the response of the fasting and non-fasting animal is a quantitative one and not a qualitative one. It is suggested that the effect of fasting upon carbohydrate tolerance should be regarded as an impaired function of the organism as a whole rather than essentially as an impairment of any one particular mechanism.

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THE EFFECT OF ERGOTAMINE ON THE BLOOD SUGAR LEVEL

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Received for publication December 13, 1928

Because of the negative sympathicotropic effect of ergotamine this drug has been used quite extensively by clinical investigators in the treatment of exophthalmic goitre. Thus Adlersburg and Porges (1), Bouchaert (2), Kliment (3), Rütz (4), Merke (5), Schönbauer, reported favorable results. A summary of their clinical results reveals that ergotamine given in doses of 0.25 mgm. to 0.3 mgm. from one to three times daily, 1, lowers the blood pressure; 2, lowers the pulse rate; 3, diminishes cardiac palpitation; 4, diminishes the fever; 5, reduces the excitability of the nervous system.

Marine, Deutsch, Cipra (7), using rabbits, noticed that ergotamine causes a striking fall in the heat production of normal rabbits without occurrence of detectable untoward effects. Abderhalden and Wertheimer (8), using rats as experimental animals, observed that ergotamine lowered the temperature of rats which were previously given thyroxin.

Flesch (9) found that the average blood sugar in Basedow's disease was as high as 170 mgm. per 100 cc., but after subcutaneous administration of ergotamine to these patients the blood sugar fell to 142 mgm. per 100 cc.

Rothlin (10) could not show a decrease in the blood sugar of normal rabbits which were given ergotamine, but if adrenalin had been given previously, ergotamine brought the blood sugar down to normal.

Lesser and Zipf (11), using ergotamine, removed the blood sugar mobilization effect of adrenalin in frog liver maintained alive by artificial circulation.

On the basis of the previous experimental and clinical work which confirmed the fact that ergotamine acts to paralyze the sympathetic and parasympathetic nerve endings, we studied the effect of ergotamine on adrenalin hyperglycemia and glycosuria of normal dogs; blood and urinary sugar of dogs which were partially pancreatectomized ($\frac{7}{8}$) and fed thyroid extract; and dogs which were totally depancreatectomized.

METHODS. The procedure in these experiments was to place the dogs

on a standard diet of 250 grams of beef heart, 225 cc. of milk, and 50 grams of bread. This diet, sufficient for their caloric need, was not changed during the course of the experiment. Total output of urine per 24 hours was recorded and samples taken for daily analysis. Toluol was used as a preservative to minimize bacterial decomposition. At the time the urine samples were taken 5 to 6 cc. of blood were withdrawn from the dog's saphenous vein before the ingestion of the standard meal and put in tubes containing potassium oxalate as an anti-coagulant.

To test qualitatively for urinary sugar the Fehling or Benedict tests were used. If the tests were negative the urine samples were discarded; if positive the amount was determined by the Folin-Wu method.

For blood sugars, the Folin-Wu quantitative method was also used, two samples of blood from each dog were run daily and the average being recorded as expressing the blood sugar level for that day.

Control experiments were run on the animals for three days or upward before preparing them for the initial experiments.

In performing a partial pancreatectomy, approximately seven-eighths of the pancreas was removed, the remnant being connected to the duodenum through the duct of Santorini. In the total pancreatectomy the duct of Santorini and Wirsung was ligated, and the pancreas stripped off, care being taken not to leave any remnant.

The ergotamine tartrate was dissolved in 0.9 per cent NaCl. One cubic centimeter of this solution was equivalent to 1 mgm. of ergotamine.

Injections of $\frac{1}{4}$ mgm. to $2\frac{1}{2}$ mgm. per kilo were given intramuscularly to dogs, the dosage being regulated by the effect the ergotamine had on the dogs. If the given dose was too high, signs of nausea, vomiting, and depression appeared. When this occurred smaller doses were given on succeeding days.

In studying the effect of ergotamine on the adrenalin hyperglycemia and glycosuria, two dogs of approximately equal weight were taken, and $2\frac{1}{2}$ cc. of 1:1000 adrenalin chloride were injected subcutaneously. The injection was given to the animals when they were in a fasting state. Ergotamine was given in some cases before the adrenalin injection, and in some instances after the adrenalin; in each case controls were run on the adrenalin hyperglycemia and glycosuria without an ergotamine injection. Two determinations were made hourly on the blood and urinary sugar by the Folin-Wu method.

To induce a hyperthyroid state, one gram of desiccated thyroid per kilo body weight, as recommended by Kunde (12), was tried and found to have the desired effect. The desiccated thyroid was mixed with the animal's food.

RESULTS. 1. *Effect of subcutaneous injection of 2½ cc. of 1:1000 adrenalin into dogs which were previously injected with ergotamine tartrate.*

	BLOOD SUGAR		URINE SUGAR	
	Low	High	Low	High
Injection of adrenalin	81	225	1.0 gm.	2.5 gm.
Injection of ergotamine tartrate followed by adrenalin	89	140	neg.	neg.

Since ergotamine paralyzes the sympathetic nerve endings, the adrenalin hyperglycemia and glycosuria are prevented. These results obtained on dogs corroborate the work of Rothlin (13) who made the same observations in rabbits.

2. *The effect of intramuscular injection of ¼ mgm. to 1¼ mgm. per kilo of ergotamine on the blood sugar of normal dogs.*

	BLOOD SUGAR			URINE SUGAR	
	Average	Low	High	Low	High
Control period	84	80	86	neg.	neg.
Injection of ergotamine tartrate for 15 days	83	69	96	neg.	neg.

Whereas our normal readings of the blood sugar during the control period were from 80 mgm. to 86 mgm., after the injection of ergotamine we obtained one blood sugar reading as low as 69 mgm. This low value as well as others obtained on different animals leads us to suspect that ergotamine lowers the blood sugar of normal dogs but not usually to a point approaching a persistent hypoglycemia.

3. *Effect of ergotamine on the blood and urinary sugar of partially pancreatectomized dogs.*

	BLOOD SUGAR			URINE SUGAR	
	Average	Low	High	Low	High
Control period	86	80	96	neg.	neg.
Post-operative control period	89	80	100	neg.	neg.
Period of injection of ergotamine tartrate 17 days	80	67	90	neg.	neg.

These results show that ergotamine lowers the blood sugar when given to partially pancreatectomized dogs. These animals respond as do the normal dogs.

4. *The effect of ergotamine on the blood and urinary sugar, pulse, and temperature of dogs partially pancreatectomized and fed thyroid extract in the dosages of one gram per kilo.*

	BLOOD SUGAR			URINE SUGAR		PULSE	TEMPERATURE
	Average	High	Low	Low	High		
							°F.
Control period	84	81	91	neg.	neg.	81	101.5
Post-operative 1 gm. per kilo thyroid extr. (19 days)	162	80	201	160 mgm.	2.6 gm.	123	103.4
Control diet without thyroid extr. (13 days)	131	84	205	1.2 gm.	1.8 gm.	115	102.2
Fed thyroid extr. (7 days)	169	90	199	1.3 gm.	1.9 gm.	128	103.1
Fed thyroid extr. and gave 1–1½ mgm., kilo ergotamine (10 days)	134	120	147	neg.	neg.	114	102.3
Fed thyroid extr. without ergotamine (6 days)	165	120	202	1.0 gm.	2.5 gm.	132	103.1

As we had shown in a recent paper (17), the feeding of desiccated thyroid extract to dogs which had been partially pancreatectomized produces a hyperglycemia and glycosuria. The effect of ergotamine (as our results show in the preceding table) on this co-existing hyperthyroidism with hyperglycemia and glycosuria, is a reduction in the blood sugar level and an elimination of the glycosuria. That the temperature and pulse are also lowered during this stage confirms the clinical investigations made on Basedow patients treated with ergotamine.

5. *Effect of ergotamine on totally depancreatectomized dogs.*

	BLOOD SUGAR			URINE SUGAR	
	Average	Low	High	Low	High
Control period	86	81	91	neg.	neg.
Post-operative period	198	187	215	1.3 gm.	4.8 gm.
Control diet + ergotamine	223	200	256	1.9 gm.	6.6 gm.

Ergotamine has no effect on the hyperglycemia and glycosuria of dogs which have been totally depancreatectomized. The dogs become progressively emaciated, and exitus took place in sixteen days following the pancreatectomy. In fact the blood and urinary sugar seem to increase following the administration of ergotamine.

SUMMARY AND DISCUSSION. Ergotamine does not effect a constant lowering of the blood sugar of normal dogs. We have in some instances

obtained lower values than our lowest normal control, but the average blood sugar during the control period, and during the periods of injections with ergotamine do not vary.

The mechanism of a thyroid hyperglycemia and glycosuria is as yet not entirely understood. Several theories have been suggested.

Falta (13) suggested that excess thyroid secretion stimulates the chromaffin tissue. In favor of this view he refers to the observations of Kraus, Friedenthal and Fraenkel (14) who detected an increase in adrenalin in the blood of patients with Basedow's disease.

Asher and Black (15) have attempted to show that the thyroid produces an increase in the excitability of those tissues susceptible to the action of adrenalin.

Opie (16) is inclined to the view that glycosuria is due to an associated lesion in the pancreas; and such lesions have been described in autopsy material.

While many clinical observations on patients with Basedow's disease showed a heightened blood sugar, our experiments dealing with experimental hyperthyroidism revealed a hyperglycemia and glycosuria only in the dogs which had the major part of their pancreas removed.

The action of ergotamine, a powerful sympathetic depressant, on the blood sugar of adrenalinized, and partially pancreatectomized dogs fed with thyroid extract (as shown in protocols I, III) showed a striking similarity, namely, a lowering of the blood sugar to normal, and an elimination of glycosuria. The discrepancies in the blood sugar of these two groups of animals may be due to the fact that in one group of animals, part of the pancreas was removed, thus reducing the factor of safety. These results also tend to indicate the mode of action of the hyperglycemia, both of the thyroid and adrenalinized dogs, namely, by way of the sympathetic system; for ergotamine, a negative sympatheticotropic substance, effectively blocked the development of a hyperglycemia and glycosuria in both instances. However, in our thyroid dogs we introduced the factor of a partial pancreatectomy in order to get an elevation in the blood sugar; for we have shown in a previous paper (17) that to obtain a hyperglycemia in dogs which were fed thyroid extract, a partial pancreatectomy had to be resorted to. This work confirms the views of Opie (16), namely, that a pancreatic lesion must exist before the development of a hyperglycemia is made possible.

Though ergotamine lowered the blood and urinary sugar of our hyperthyroid and adrenalinized dogs, it had no similar effect in completely pancreatectomized dogs. Injections of ergotamine in amounts similar to those given to other dogs did not cause a fall in the blood sugar. The animals rapidly became cachectic and progressively emaciated. Exitus took place in sixteen days.

From the results obtained it is apparent that though ergotamine diminishes sugar mobilization in case of adrenalinized and hyperthyroid dogs, it had no effect on sugar utilization in dogs which had been totally depancreatectomized.

CONCLUSIONS

On the basis of our work on dogs with ergotamine we can state that:

1. It causes a variable lowering of the blood sugar of normal dogs, but does not affect the average blood sugar during the period of its adminstration.

2. It prevents the development of an adrenalin hyperglycemia and glycosuria.

3. It lowers the blood sugar and eliminates the glycosuria in dogs which have been partially pancreatectomized and fed thyroid extract.

4. Ergotamine has no effect on the hyperglycemia and glycosuria of dogs which have been made diabetic by a total pancreatectomy.

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THE REGULATION OF RESPIRATION

XXVI. Total Carbon Dioxide Content of the Brain as Affected by Hemorrhage and the Injection of Sodium Cyanide

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Received for publication December 17, 1928

It is known that $NaHCO_3$ consitutes an important part of the buffer mechanism of the human body. In conditions in which there is a production of a non-volatile acid, the sodium is drawn away from the $NaHCO_3$ and the acid—lactic, suphuric, and aceto-acetic, or other acids are excreted with the sodium through the kidney and intestine, except in those instances where there is a return to the precursor state. In this way there is a depletion of the available bicarbonate. Such changes are reflected in the CO_2 content of the blood.

Plasma has been shown to yield 50 to 65 vol. per cent of CO_2 (Van Slyke and Cullen, 1917; Joffe and Poulton, 1920). McClendon (1918) has shown the spinal fluid to yield 58 to 63 vol. per cent of CO_2. When one turns to the literature for values of tissue alkalinity it is found that until recently this question has gone largely unheeded. Such facts are however highly desirable in any effort at explaining respiratory control. We would, in other words, consider a study of the total carbon dioxide content of brain tissue as a logical step in the elucidation of the control of respiration in view of the fact that acidity of the respiratory center itself may be a factor in the control of respiration, and that the respiratory center possesses an acid metabolism of its own (Gesell, 1923). That this metabolism varies considerably in the brain with anaerobiosis has been shown by McGinty and Gesell (1925) who have demonstrated that lactic acid accumulates rapidly, and more recently McGinty (1928) has shown that lactic acid is eliminated from the brain into the blood stream under less extreme impairment of oxidation. It should follow that the labile mechanism which buffers the acid metabolism would in conditions of impaired oxidation be reduced to a lower level. It was thought that an analysis of the brain for total carbon dioxide content before and after a period of impaired oxidation might throw light on this question.

Method. Our procedure was arranged to show first, the normal CO_2 content of the brain and blood; second, the CO_2 content of the brain and blood after hemorrhage, and third, the CO_2 content of the brain and blood after the injection of sodium cyanide.

Small dogs ranging in weight from 3 to 6 kilos were used. Blood samples were taken from the femoral artery and vein and the plasma analyzed according to the method of Van Slyke.

Brain tissue was obtained by means of a T-shaped guillotine. The blades are so arranged to sever the head from the body and split the skull in half with a single blow. Tissue obtained from the larger animals was divided into four different anatomical parts; the two halves were used in the experiments with the small animals. This tissue was immediately placed in specially constructed 300 cc. stoppered beakers. This was done on an average within two minutes. To avoid autolysis and loss of CO_2 we have placed the samples in 20 cc. of cold 0.1 N NaOH. Four hundred milligrams of NaF and two drops of caprilic alcohol were added to further inhibit autolysis and prevent foaming, respectively. However, from this it must not be inferred that we have entirely prevented chemical reaction. It was found early in the investigation that, whereas NaOH prevented the loss of CO_2, it also caused an increase in the amount of CO_2 already present. It was found that samples kept at 5°C. showed a slower rise in CO_2 content. As compared with a 10 per cent increase in twenty-four hours in these samples a 31 per cent increase was found in those samples kept at room temperature. Inasmuch as analyses were made shortly after decapitation the conclusions of our experiment would not appear to be affected by these findings.

Our efforts to break up the tissue under carbon dioxide free conditions have led us to try various methods, but with the arrangement shown in figure 1 we have obtained the best results.

With this method we are able to convert a 25 gram sample to a fine brei without the loss of CO_2 and within a period of 4 minutes. If necessary as much as 30 grams can be ground, and with careful technique the tissue can be analyzed using the Van Slyke apparatus within 25 minutes following removal of the tissue. The brei is taken from the grinder and poured into 250 cc. glass stoppered Erlenmeyer flasks kept at 5°C. and from this 1 cc. samples were taken as aliquot parts for analysis. To reach complete equilibrium the samples were shaken for a period of 5 minutes. Results were determined on the basis of cubic centimeters of CO_2 per 100 grams of tissue.

Results. Total CO_2 of brain and blood as determined from the analysis of tissue and plasma taken from normal anesthetized animals was as follows: the average value for tissue, 48.98 vol. per cent; that for arterial blood plasma, 58.58 vol. per cent. These values maintained an average ratio (Br./Bl.) of 0.84 (see table 1).

With hemorrhage (table 2) variations occur in the total CO_2 content of brain. A maximum amount of 39.90 vol. per cent and a minimum of 28.43 vol. per cent was found.

The injection of 0.01 N NaCN reduced the total CO_2 in brain to 19.42 vol. per cent. In experiment 4C although 95 cc. of 0.001 N.NaCN was injected the total CO_2 content of the brain remained at a level near that found in normal animals while the blood value fell to 42.79 vol. per cent.

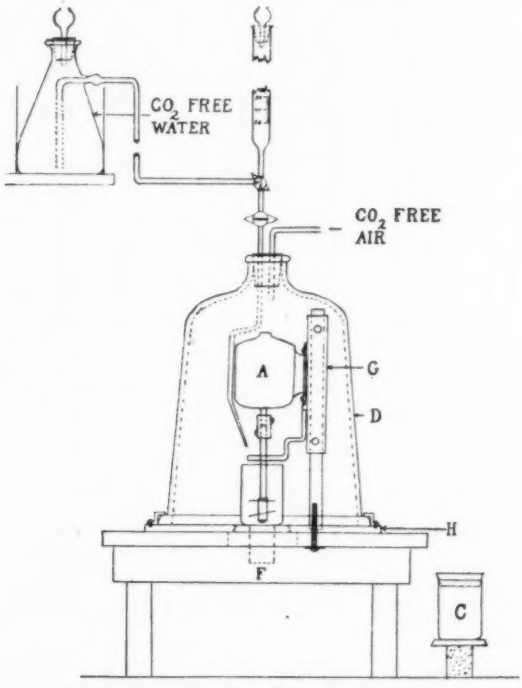

Fig. 1. (Carbon dioxide free tissue grinder.) *A* represents a small high speed motor ($\frac{1}{20}$ H.P., R.P.M. 1750) mounted on a sliding shaft, *G*, which permits a raising or a lowering of the motor to any desired height. Screw clamps hold it in place. To the end of the motor shaft is fastened a second shaft by means of a union on which are mounted two blades arranged as shown. The motor was arranged so that the end of the shaft remained about $\frac{1}{8}$ inch from the bottom of the beaker which is fastened in place in the aperture in the hard rubber base, *H*. Covering both motor and shaft is a large bell jar mounted air tight on the rubber base. CO_2 free air is admitted through a valve in the opening at the top of the bell jar. The content of the chamber was changed before each sample was ground. Another tap admits CO_2 free water which is used to dilute the brei in cases where large amounts of tissue are ground. The motor was under rheostatic control.

The injection of 0.05 N NaCN produced relatively similar results as shown in experiments 5, 6, 7C (see table 3).

Discussion. The results of these experiments indicate the approximate

TABLE 1

Normal animals

EXPERIMENT NUMBER	DATE	WEIGHT OF DOG	BLOOD (SERUM) (SAMPLE TAKEN JUST BEFORE DECAPITATION)		BRAIN				K Br/Bl.	REMARKS
			Ven.	Art.	Kind	Alk. res.	Av.	Var.		
		kilos	*vol. per cent*	*vol. per cent*		*vol. per cent*	*vol. per cent*	*vol. per cent*		
1A	12/31/27	6.2		56.05 56.03 56.93	Cereb. Med. Cereb. Mid. br.	45.17 49.14 53.08 49.74	49.28	+0.33	0.90	Four arterial blood samples were taken at 5 minute intervals, the last one being taken just before placing the animal under the guillotine
2A	2/ 4/28	3.5		60.25	Cereb. Med. Cereb. Cereb.	46.58 44.44 48.74 42.26	45.50	−3.489	0.75	Three arterial samples were taken at 5 minute intervals: 1, 61.55 vol. per cent; 2, 60.89; 3, see col. 5
3A	2/10/28	6.0		51.88	Cer. Cer. Cer. Mid. br. Med.	49.33 56.60 53.89 43.41 39.18	47.88	−1.11	0.90	Four blood samples, 2 venous and 2 arterial were taken at 5 minute intervals: 1, 56.44; 2, 55.938; 3, 50.778; 4, see col. 5
4A	2/12/28	5.0		58.48	L. half R. half	51.31 56.99	54.15	−5.16	0.93	Only one arterial sample taken
5A	2/17/28	6.1		60.67	Cer. Cer. Mid. br. Medulla	44.84 59.56 44.45 46.74	49.14	+0.15	0.81	Two arterial samples taken just before decapitation: 1, 60.20 vol. per cent; 2, see col. 5
7A	2/21/28	4.0		58.47	Cer. Cer. Cer. Med.	43.30 67.49 39.78 43.98	48.63	−0.36	0.83	Four arterial samples were taken, two being taken 10 minutes before decapitation, and two 5 minutes minutes later: 1, 60.71; 2, 60.22; 3, 60.03; 4, see col. 5
8A	5/12/28	4.2		64.29	L. half R. half	46.13 50.605	48.36	−0.63	0.75	One arterial sample only was taken just before decapitation

TABLE 2

Hemorrhage

EXPERIMENT NUMBER	DATE	WEIGHT OF DOG	AMT. OF HEM.	BEGIN. HEM.	END OF HEM.	T. OF DECAP.	BLOOD (SERUM)		BRAIN				K	REMARKS
							Ven.	Art.	Kind	Alk. res.	Av.	Var.		
							vol. per cent	*vol. per cent*		*vol. per cent*	*vol. per cent*	*vol. per cent*		
2B	2/25/28	5.1	145	10:40	11:15	11:40		35.44 32.66	L. half R. half Med.	36.46 24.41 24.43	28.43	−5.5	0.87	Animal anesthetized at 8:15. Arterial sample from the normal animal yielded 67.80 vol. per cent (female A) 80 cc. hem.—10:40; 25 cc. hem. at 11:00; 20 cc. hem. at 11:15
3B	3/ 1/28	3.2	98	2:10	2:17	3:12		43.01	L. half R. half	31.48 30.83	31.15	−2.8	0.75	Normal arterial sample yielded 53.529 vol. per cent CO_2
4B	3/ 6/28	6.5	150	2:00	2:08	2:46			Cer. Cer. Mid. b. Med.	39.75 34.18 44.37 41.32	39.90	+5.9		Arterial samples from the normal animal yielded 53.384 vol. per cent CO_2, animal anesthetized at 8:30 a.m.; experiment begun 2:46 p.m.
6B	3/13/28	5.0	100	1:32	1:37	2:50		43.47	Cer. Cer. Mid. Med.	43.21 25.28 35.93 44.36	37.19	+3.2	0.86	Venous blood samples from the normal animal yielded 59.02 vol per cent CO_2 arterial samples yielded 60.44 vol. per cent; arterial sample at end of hem.—53.44 vol. per cent
7B	3/21/28	6.2	125	11:04	11:10	11:46			Cer. Cer. Mid. Med. Cer.	36.72 38.00 33.49 32.58 41.55	36.46	+2.4		Arterial blood from the normal animal yielded 61.26 vol. per cent; venous blood from the femoral vein yielded 61.68 vol. per cent CO_2
8B	3/31/28	6.5	150	8:20	8:28	9:47			Cer. Cer. Mid.	39.01 26.77 26.44	30.74	−3.2		Arterial blood from the normal animal yielded 61.26 vol. per cent; venous blood from the femoral vein yielded 61.68 vol. per cent CO_2

TABLE 3

Injection of NaCn

EXPERIMENT NUMBER	DATE	WEIGHT OF DOG	NORM. OF NaCN	AMT. INJECTED	TIME OF INJECTION	TIME CONSUMED IN INJECTING	TIME OF DECAP.	BLOOD (SERUM)	BRAIN				K	REMARKS (Note: All blood samples taken from femoral artery and vein)
								Arterial	Kind	Alk. res.	Av.	Var.		
		kilos	*N*	*cc.*		*min.*		*vol. per cent*		*vol. per cent*	*vol. per cent*	*vol. per cent*		
3C	3/17/28	4.0	0.01	14	10:21 10:30 10:44	1:41 1 1:45	10:51	38.598	Cer. Med. Cer. Mid. b.	20.06 25.89 15.64 16.11	19.425	−16.0	0.50	Normal arterial sample yielded 60.73 vol. per cent; normal venous sample yielded 59.57 vol. per cent; sample after second injection—(venous) 46.43 vol. per cent; sample taken just before last injection yielded 44.14 vol. per cent (venous); arterial sample after second injection yielded 42.09 vol. per cent; arterial sample taken during apnea following second injection yielded 43.71 vol. per cent
4C	5/24/28	6.5	0.001	95	11:22	1:25 3:41 5:63	12:05	42.795	Cer. Cer. Mid. b.	45.41 38.97 49.93	44.77	+8.9	1.04	Normal venous sample yielded 65.53 vol. per cent; venous sample 10 minutes before decapitation yielded 42.873 vol. per cent; arterial sample 2 minutes before decapitation yielded 42.795 vol. per cent
5C	4/18/28	3.4	0.05	25	3:37	3:58	4:22	45.32	L. half R. half	35.80 36.56	36.18	+0.32	0.80	Normal arterial sample yielded 60.69 vol. per cent
6C	4/24/28	3.5	0.05	25	2:40	2:51	3:22	48.80	L. half R. half	32.82 35.22	34.02	−1.8	0.70	Normal arterial sample yielded 58.23 vol. per cent; arterial sample taken 5 minutes before decapitation yielded 49.55 vol. per cent
7C	4/27/28	3.5	0.05	19	3:46	3:51	4:00	70.05	L. half R. half	36.75 39.45	38.10	+2.2	0.54	One sample only taken in this experiment (7C)
8C	5/ 9/28	4.1	0.05	25	2:32	2:35	2:42	37.984	L. half R. half	40.27 45.17	42.72	−6.86	1.13	Normal arterial sample yielded 55.317 vol. per cent

bicarbonate content of mixed brain tissue under fairly normal conditions. They show further that this content may rapidly fluctuate with disturbances in tissue oxidation. To what extent the reduction in total carbon dioxide content is due to formation of fixed acids accompanied by a driving off of carbon dioxide, and to what extent it is due to the blowing off of carbon dioxide has not yet been definitely determined. That a large part of the reduction is a function of the accumulation of lactic acid is clearly indicated by the experiments of McGinty and Gesell (1925). They not only showed that lactic acid accumulated in the brain of the decapitated dog at a rate of 20 mgm. per 100 grams of tissue per minute but also showed that the brain of an animal decapitated after prolonged exposure to carbon monoxide contained 48.10 mgm. more lactic acid than the normal brain. More recently McGinty (1928) has shown that the lactic acid content of the venous blood leaving the brain is increased by occlusion of the head arteries and by the intravenous injection of sodium cyanide.

It is fair then to assume that the buffer base of the respiratory center is diminished by impaired oxidation in this tissue. This change in itself must mean increased hydrogen ion concentration of the center. Assuming that the hydrogen ion concentration of the respiratory center rather than that of the blood is the more significant factor in the control of respiration the explanation of increased ventilation from hemorrhage and intravenous injection of sodium cyanide by Gesell, Capp and Foote (1922), McGinty and Gesell (1927), Hertzman and Gesell (1927), Hertzman and Gesell (1928) is substantiated. Assuming that the respiratory center is stimulated by the formation of lactic acid in its cells rather than by its appearance at the center in the blood the initial increased alkalinity of the blood from excessive ventilation with hemorrhage and the administration of cyanide may be accounted for. But inasmuch as lactic acid accumulation within the tissue means migration of acid into the blood the buffer base of the blood is decreased as well. In time this acid effect may predominate and eventually lead to a hydrogen ion concentration of the blood above normal. The increasing amount of lactic acid in the blood with hemorrhage as found by Bennet (1926), McGinty (1928), Gesell, Bernthal, Gorham and Krueger (1928) and following the administration of cyanide, by Gesell, Bernthal, Gorham and Krueger (1928) and McGinty (1928), support this view.

Inasmuch as increased ventilation means increased blowing off of carbon dioxide, it cannot be said without further experiments what change in reaction occurs within the brain as a result of accumulation of fixed acids during impaired oxidations. To arrive at such information it will be advisable to establish carbon dioxide dissociation curves of the intact brain with varying disturbances in oxidation.

SUMMARY AND CONCLUSIONS

A method of preparing brain tissue for the analysis of total carbon dioxide has been devised.

Total CO_2 content of the brain has been studied in normal animals, hemorrhaged animals, and in animals following cyanide injection. Normal ventilation has been used in each series of experiments.

Values for the normal animal taken from seven different experiments showed an average of 48.98 vol. per cent of CO_2 within the brain and 58.58 vol. per cent CO_2 in the plasma of the arterial blood.

Conditions of lowered oxidation were found to produce a falling off in the total CO_2 within the brain. With hemorrhage in six different experiments the results ranged from 39.90 vol. per cent as a maximum to 28.43 vol. per cent as a minimum. With the injection of NaCN the results varied from 44.77 vol. per cent to 19.42 vol. per cent.

These results indicate that the buffering capacity of the normal brain is less than that of the normal blood and that the buffering capacity of both is reduced with impaired oxidation.

These data are in accord with the view that the hydrogen ion concentration of the center is an important factor in the control of respiration. This has been alluded to in the discussion.

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EXPERIMENTAL BONE MARROW REACTIONS

VI. The Adequacy of Kidney, Pancreas, Spleen and Brain for Blood Regeneration in Pigeons with Nutritional Anemia Produced by Starvation

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Received for publication December 29, 1928

An unusual opportunity for studying the influence of various kinds of food on blood regeneration presents itself in pigeons, for in them bone marrow aplasia and hence anemia can be produced in a physiological manner by simple withdrawal of food. The method is comparable to in *vitro* cultures, with this important difference, that the growth of bone marrow cells in starved pigeons takes place in its natural environment. As in tissue cultures, one may have, theoretically, media surrounding the bone marrow cells which will or will not support growth. Differences in response may be due to some deficiency or excess in the material supplied. In an otherwise adequate medium, substances which either inhibit and stimulate growth, may produce a result because of a certain balance between these opposing forces.

That conditions for growth of various cells are not identical has been brought out by the extensive work on in *vitro* cultures. In *vivo*, dissociation of general body growth and blood regeneration has been demonstrated by Hart and associates (1). They fed young rabbits on whole milk and thus produced a severe anemia without interference in the gain of body weight. The increase in weight did not continue indefinitely, however, because when the hemoglobin content reached 50 to 55 per cent, growth ceased and death supervened.

In a series of experiments on pigeons fed broiled beef liver (2a) after a period of starvation, this dissociation of body weight and blood regeneration was confirmed. The pigeons fed broiled liver regained their weight rapidly, while the erythrocytes and hemoglobin, after an initial increase to a point below normal, declined steadily, until an anemia, equal in magnitude to the anemia at the end of the starvation period, had been produced. This anemia was concomitant with a definite lack of megaloblasts in the bone marrow. That the results obtained in pigeons with broiled liver feeding were not due to a deficiency in liver as a food, was demonstrated by feeding liver which had been extracted with 45 per cent ethyl alcohol

(2b). With this extracted liver, blood regeneration progressed in a normal manner, the replacement of body weight being relatively slow but adequate. The results obtained with broiled liver, therefore, were interpreted as being due to some substance or substances in the whole liver which in excess was inimical to blood formation.

It was though worth while to observe the effect of feeding other organs, on the replacement of weight, red blood cells, and hemoglobin in starved pigeons. This paper deals with the results obtained in four series of pigeons fed respectively prepared beef kidney (group I), pancreas (group II), brain (group III), and spleen (group IV).

EXPERIMENTAL OBSERVATIONS. The experimental conditions and procedures were identical with those employed in a previous study (2a). Before feeding the kidneys, pancreas, and spleens were broiled, while the brain was first broiled, then subdivided and air dried. The food was always supplied in excess of the amount actually consumed. The general course of the different groups is illustrated in the table.

This table gives three sets of data, namely, the weight, number of erythrocytes per cu. mm., and the per cent of hemoglobin 1, at the beginning of the period of starvation, representing apparently normal values; 2, at the end of the starvation period, and 3, at the end of the period of feeding.

I. The feeding of broiled kidney. The results in this group of 12 pigeons were variable, a few regained their weight, red blood cells, and hemoglobin, while others were definitely anemic at the end of the period of feeding. The average weight of this group, exclusive of no. 52 and no. 36 fed for only four and twelve days respectively, which is insufficient time for the return to normal with a favorable diet, shows that the average weight of this series of pigeons was almost regained. The initial average weight before starvation was 326 grams, the final average weight 321 grams. The highest average weight during the period of feeding was 328 grams, indicating that as a group the initial weight was regained, followed by an insignificant loss.

In the pigeons fed broiled kidney the initial number of erythrocytes averaged 3.5 millions per cu. mm. and the hemoglobin 95 per cent. At the end of the period of feeding the red blood cells averaged 2.9 millions per cu. mm. and the hemoglobin 75 per cent,—considerably lower values than the initial determinations. The lowest averaged count of the red blood cells during the period of feeding was 2.2 millions per cu. mm., and the lowest averaged hemoglobin was 56 per cent, while the highest average for the erythrocyte count was 3.3 millions per cu. mm. and for the hemoglobin percentage was 91. In comparing these figures with the final averages 2.9 millions per cu. mm. for the red blood cells and 75 per cent for the hemoglobin, it appears that this group of pigeons taken as a whole did not wholly regain their red blood cells and hemoglobin during the

TABLE 1

NUMBER	INITIAL WEIGHT	INITIAL NUMBER OF R. B. C.	INITIAL HGB.	DAYS OF STARVATION	LOSS OF WEIGHT	R. B. C. AT END OF STARVATION	HGB. AT END OF STARVATION	NUMBER OF DAYS FED	FINAL WEIGHT	LOSS OR GAIN OF WEIGHT	FINAL NUMBER OF R. B. C.	FINAL HGB.
					Kidney							
	grams	*millions per cu. mm.*	*per cent*		*grams*	*millions per cu. mm.*	*per cent*		*grams*	*grams*	*millions per cu. mm.*	*per cent*
52*	423	3.4	91	17	111	3.0	83	4	340	−83	2.6	79
36*	396	3.9	92	10	96	2.7	80	12	369	−27	2.6	76
39	327	3.0	99	13	101	2.1	66	21	313	−14	2.9	92
34	297	3.8	82	15	103	2.5	50	24	301	+4	2.6	66
38	332	3.3	92	10	117	2.8	63	38	338	+6	2.9	69
32	267	3.9	89	10	54	3.1	87	39	199	+68	2.2	63
78	416	3.7	118	13	166	2.7	69	39	456	+40	3.3	87
76	262	3.8	109	10	102	2.9	79	42	294	+32	3.2	83
94	277	3.7	120	12	104	2.6	78	49	320	+43	3.4	102
33	272	3.9	82	10	72	3.4	78	54	201	−71	2.1	46
35	392	2.9	83					51	349	−43	2.6	63
37	420	2.9	92					54	436	+16	3.9	83
Av.	340	3.5	95		102.6	2.7	71		321	−19	2.9	75
					Pancreas							
51	279	3.0	78	12	108	2.8	71	25	287	+8	3.3	87
53	386	3.0	80	12	112	3.1	79	25	371	−15	3.4	76
98	350	3.2	112	13	119	2.5	81	39	359	+9	3.7	92
82	458	3.8	105	20	191	2.9	83	46	470	+12	3.6	104
80	404	3.7	105	15	151	2.8	89	47	404		3.5	95
Av.	375	3.3	96		136	2.8	80.6		378	+3	3.5	90.8
					Spleen							
125	414	4.2	108	42	224	2.8	80	20	289	−125	3.6	106
122	408	3.6	114	37	213	2.3	78	25	281	−127	3.8	102
117	383	4.0	99	34	206	2.7	78	33	275	−108	4.3	127
120	325	4.0	104	14	112	2.8	83	50	294	−31	4.4	125
Av.	382	3.9	106		188	2.6	79.7		284	−98	4.0	115
					Brain							
108	383	4.3	109	33	175	2.9	83	17	245	−138	3.1	89
50	342	3.9	83	21	96	2.8	64	19	274	−68	2.5	66
49	344	3.4	83	12	123	1.5	48	28	275	−69	2.2	60
Av.	356	3.8	91.6		131	2.4	65		264	−92	2.6	71.6

* Not included in averages.

period of feeding, and that the final values for these constituents averaged lower than the highest values obtained, indicating that there was a final decline.

II. The feeding of broiled pancreas. This series of 5 pigeons regained their weight, red blood cells, and hemoglobin. The final average of the hemoglobin, 90.8 per cent, is within normal limits although slightly lower than the average initial hemoglobin of 96 per cent. The highest average figures obtained during the period of feeding were for weight 379 grams, for red blood cells 3.6 millions per cu. mm., and for hemoglobin 98 per cent.

III. The feeding of broiled spleen. The striking feature about this group of 4 pigeons was the excellent regeneration of red blood cells and hemoglobin. The average of the lowest counts of the red blood cells was 2.5 millions per cu. mm., and the lowest hemoglobin percentages averaged 71. The averages of the high values for both red blood cells and hemoglobin were practically identical with the average final values of 4.0 million erythrocytes per cu. mm. and 115 per cent hemoglobin, which are to be compared with the initial values of 3.9 millions erythrocytes per cu. mm. and 109 per cent of hemoglobin. The absolute loss of weight in some of these large starved pigeons was considerable. The relative loss of body weight in 3 out of the 4 animals fed spleen was 52, 54, and 55 per cent of the initial body weight, and in one, 34 per cent as compared with the range of 20 to 43 per cent in the other groups. Nevertheless, the results seem to indicate that spleen feeding was less favorable for the replacement of lost body weight than pancreas. In one animal which had lost 112 grams or 34 per cent of the initial body weight, only 81 grams or 72 per cent of the lost weight were replaced during 50 days of spleen feeding.

IV. The feeding of broiled and dried brain. The table shows that the average final weight, number of red blood cells, and percentage of hemoglobin of the 3 animals fed brain were only slightly above the values obtained at the end of the starvation period. The average of the highest figures obtained were for weight 284 grams, for erythrocytes, 2.8 millions per cu. mm., and for hemoglobin 77 per cent,—values only slightly above the final ones, indicating that the animals merely existed on this type of food. The stools throughout were voluminous, light in color and very foul.

The microscopical picture of the bone marrow of normal pigeons, as well as of pigeons fed grain after a period of starvation, has been described previously (2a). The main features are a moderate hyperplasia throughout the femoral bone marrow; the radial bone marrow is less hyperplastic with the cellularity confined mainly to the periphery. The number of immature erythrocytes averages about 50 per cent of the total number of cells. The immature forms of the erythrocytes are confined in definite islands, consisting of a mixture of erythrocytes of various ages, with the

more mature cells in the center of the island and the youngest cells of the erythrocytic series, or the megaloblasts, about the edges. This picture of the bone marrow occurs when there is an adequate supply of red blood cells and hemoglobin in the peripheral blood.

The bone marrow architecture of the pigeons fed broiled kidney after a period of starvation varied more than that of any of the other 3 groups kept under comparatively constant conditions. Thus in three of the animals (nos. 32, 76, 78) the bone marrows were more hyperplastic than normal; the red blood cell islands were small and contained fewer megaloblasts and young cells than in the controls. That is, the picture was similar to the one obtained in animals fed exclusively on liver (2a) although less marked. In three other pigeons (nos. 37, 39, 94) the bone marrow was classified as normal, while in the remainder the bone marrow was very hyperplastic with red blood cell islands containing more immature cells than found in the controls. The proportion of red cells to white cells in the bone marrows of this group, fed broiled kidney, was determined by supravital counts. The erythrocytes ranged from 40 to 71 per cent. No correlation could be made between the proportion of the erythrocytes in the bone marrow and the peripheral blood, except that in no. 33 with the lowest number of red blood cells per cu. mm., there was only 40 per cent of cells belonging to the erythrocytic series in the bone marrow.

The bone marrows of the 5 pigeons fed broiled pancreas showed a more consistent picture. As compared with normal, the hyperplasia was more extensive, with erythrocytic centers of normal composition predominating. The percentage of cells belonging to the erythrocytic series was determined in two animals only (nos. 51 and 53) and was found to be 63 and 64 per cent respectively.

The main feature of the bone marrows from the 4 animals fed spleen was the moderate cellularity, which, in some instances, seemed to be less than normal, and the varying age of the erythrocytic centers. Many of the blood islands were composed chiefly of megaloblasts; others had a normal composition while some consisted of practically only mature cells. This corresponds to an adequate or more than adequate supply of red blood cells and hemoglobin in the peripheral blood, and it suggests that the efficient marrow was not excessively hyperplastic.

In two cases the pigeons fed brain showed aplasia of the radial marrow, while in no. 108, fed for 17 days, the bone marrow was practically normal.

In the pigeons fed broiled liver (2a), fatty infiltration of their liver was a constant feature. Six of the 12 pigeons fed broiled kidney showed fatty infiltration of the liver, in three slight, in one moderate, and in two extensive (nos. 37 and 32). Among the 5 pigeons fed pancreas, four showed fatty infiltration of the liver, one moderately and three extensively (nos. 80, 82 and 98), while the liver of two of the four fed spleen showed exten-

sive fatty infiltration (no. 117, 122). There was no fatty infiltration of the liver in the brain fed group. By correlating the findings of fatty infiltration of the liver and the efficient function of the hematopoietic organs, it is evident that extensive fatty infiltration of the liver does not interfere with blood formation during the period of the experiments here considered.

The livers, spleens, and bone marrows were examined for iron. In the controls fed grain, with or without a period of starvation (2a), there were considerable amounts of iron, in both the liver and the spleen, with small amounts occasionally in the bone marrow. In the group fed kidney, the iron content of the tissues varied from moderate to considerable amounts in the liver and spleen, with no iron, as a rule, in the bone marrow. In the pigeons fed broiled kidney no correlation could be made out between the amount of iron in the tissues and the hemoglobin content of the peripheral blood. Very small amounts of iron were found in the organs of the animals fed either pancreas or brain, while in the four pigeons fed broiled spleen moderate amounts were present in the liver and spleen, but there was no iron in the bone marrow.

Discussion. It is evident that kidney, or liver, as an exclusive food is not as effective as pancreas or spleen for the replacement of red blood cells and hemoglobin in a nutritional anemia in pigeons with a partially aplastic bone marrow. This contrasts markedly with the results obtained by Whipple, Robscheit-Robbins, and associates, who found in dogs with anemia due to chronic blood loss, that liver (3), (4) and kidney (4) caused regeneration of hemoglobin more efficiently than other foods. In contrast to kidney, pancreas seems to be adequate in pigeons for the replacement of both weight and blood regeneration, and also for the maintenance of these factors during the time of the experiment. It is noteworthy that in these experiments spleen proved to be the most adequate food for the replacement of the red blood cell constituents of the peripheral blood, while less effective for the replacement of lost weight than liver, pancreas or kidney. There were only 4 pigeons fed spleen, which is not a sufficient number to enable one to draw definite conclusions, but the results obtained may be interpreted tentatively as indicating that blood regeneration and the replacement of lost body weight in the pigeon may be dissociated when spleen is fed. This dissociation, however, is the reverse of that which occurs when liver is fed pigeons (2a). In such animals the replacement of body weight was rapid in face of an insufficient blood regeneration and subsequent increasing anemia.

The effect of spleen feeding on blood formation in pigeons recalls the work, both clinical and experimental, with splenic extracts by various observers (5), (6), (7), (8), (9). These authors found that spleen or splenic extract when administered under certain circumstances causes an increase of red blood cells in the peripheral blood.

As basic foods, kidney and especially liver are less effective than spleen, in building up an adequate supply of red blood cells and hemoglobin from a comparatively aplastic bone marrow in pigeons. Yet they are eminent for the regeneration in dogs of hemoglobin removed by bleeding (3), (4). The inadequate blood regeneration in pigeons fed liver has been shown to be due to an excess of some substance in the liver fed inimical to the proper functioning of the hematopoietic organs (2b), while the variable results obtained by kidney feeding remain unexplained. Pancreas on the other hand seems to be adequate for the formation of red blood cells as well as hemoglobin, although the slightly lower values of hemoglobin in the peripheral blood indicates that perhaps insufficient amount of hemoglobin forming substances is present. Brain proved to be insufficient as a food.

The influence of substances upon building up blood cells from a partially aplastic bone marrow produced by starvation in pigeons may prove of value in elucidating the fundamental problem as to what cells, particularly red blood cells, need for adequate growth and function. The same experimental procedure can not be employed in mammals; because, unfortunately, most mammals die before any marked changes are produced in the bone marrow by starvation. The results obtained in pigeons may be shown, however, to be applicable in a broader sense to cell growth, especially if the future should prove that the biological reactions of cells similar in function react fundamentally in the same manner.

CONCLUSIONS

1. This study concerns the effect of feeding beef kidney, pancreas, brain and spleen on blood and body weight regeneration of pigeons in which partial bone marrow aplasia and thus anemia had been produced by starvation.

a. Broiled kidney feeding gave variable results. A few animals regained their weight, red blood cells and hemoglobin, while others were definitely anemic at the end of the period of feeding.

b. Pancreas proved to be adequate for the replacement of weight, red blood cells, and hemoglobin.

c. Broiled spleen feeding caused an excellent regeneration of the blood, superior to any of the organs tested. The replacement of weight, however, was slow.

d. Brain proved to be inadequate as a food.

2. The bone marrows of the pigeons fed kidney showed a variable microscopical picture. A few were normal, while others were classified as containing either more or fewer cells of the erythrocytic series than normal. No definite correlation could be made out between the peripheral blood and the findings in the bone marrow.

The bone marrows of pigeons fed pancreas were slightly more hyperplastic than normal.

The bone marrows of the spleen fed group seemed less hyperplastic than normal, indicating that this most efficient marrow did not function because of excessive hyperplasia.

Two of the three pigeons fed brain had an aplastic radial marrow, while in the other it was of a normal composition.

3. Varying amounts of fatty infiltration of the liver was found in animals from all four groups except the one fed brain.

4. The iron content of the tissues varied from moderate to considerable amounts in the kidney fed group, while very small amounts were found in the tissues of the animals fed pancreas and brain. Moderate amounts of iron were found in the spleen and liver of pigeons fed broiled spleen.

5. It is concluded that kidney as an exclusive food is not as effective as pancreas or spleen for blood regeneration in a nutritional anemia in pigeons caused by starvation, while brain is inadequate.

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THE QUESTION OF WATER REABSORPTION BY THE RENAL TUBULE AND ITS BEARING ON THE PROBLEM OF TUBULAR SECRETION

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Received for publication December 20, 1928

In a recent paper (White, 1928) determinations of the pressure in the lumen of the glomerular capsules of Necturus were reported. The pressures were found to be high enough (up to 57 mm. H_2O) to become a significant factor in the equation expressing the effective filtering head of pressure, E.F.H. = (G.C.P. − I.C.P.) − C.O.P., where E.F.H. designates effective filtering pressure head, G.C.P. glomerular capillary pressure, I.C.P. intracapsular pressure and C.O.P. colloidal osmotic pressure of the plasma. The details of these pressure relations and their bearing on the question of glomerular filtration are being further investigated.

Aside from the glomerular filtration problem it was recognized that the opportunity was available of obtaining data on the rate of passage of glomerular fluid and on tubular dimensions, of putting them into Poisseuille's equation expressing the rate of flow of a liquid through a tube, and of comparing the pressure head so calculated with the observed intracapsular pressure. The calculation of the head of pressure required to drive a liquid at a given rate through a tube of given dimensions is, of course, made with the condition that all the liquid goes all the way through the tube. If the calculated pressure head agrees with the observed intracapsular pressure the inference is that no water is being reabsorbed by the tubules, for if reabsorption were taking place to any significant extent the actual intracapsular pressure would be less than that calculated on the basis of all the water passing through the entire length of the tube. A consideration of this problem forms the topic of the present paper.

The animals were prepared for operation either by pithing the brain without an anesthetic or by immersion in 2 to 3 per cent urethane solution. Not infrequently, with either method, a poor circulation through the kidney is observed even though the circulation is quite active in other organs. In such event the animal is discarded; the next animal, treated in apparently the same way, may show an active renal circulation. The ventral surface of the kidney was exposed and was illuminated either by a

Tungarc lamp or by a carbon arc, the light in either case passing through a dilute copper sulphate filter. Ringer's solution was kept dripping over the exposed kidney surface. The pressure in the lumen of the capsule of a glomerulus exhibiting active circulation was determined by the method described in the above reference. This pipette was then removed, a mercury-filled pipette inserted and glomerular fluid collected for a ten minute period; the quantity of fluid collected was calculated from measurements of the fluid column. After removal of the pipette containing the glomerular fluid the dye-containing pipette which had been used for the measurement of intracapsular pressure was again inserted and its leveling bulb raised just above the level at which intracapsular pressure had been measured, with the result that dye was run into the capsule and down the tubule at a pressure only slightly above the observed intracapsular pressure. Any undue distention of the tubule with a resultant erroneously high diameter was thus avoided. The diameters of the various segments of the injected tubule were then measured, a 16 mm. objective and a micrometer scale with lines 0.05 mm. apart placed in an 8× ocular being used. With this optical system one scale space of the ocular micrometer subtends a distance of 8μ on the object measured. To get the lengths of the various tubule segments a camera lucida drawing was next made, using a 40 mm. objective and a 2× ocular, which gave a 30× magnification. It may be pointed out here that the microscope tubes were removed from their bases and supported on movable stands, by which arrangement any desired part of the object could be brought into the field. This was true both of the binocular microscope used for the pipette manipulations and of the monocular used for tubule diameter measurements and camera lucida drawings. This was necessary for the following reason. It is, of course, obvious that after a pipette is in a capsule the animal board cannot be moved about on the stage; the alternative is to put the tube on a movable stand so that it can be moved to any desired portion of the object. One could, of course, insert the dye pipette into a capsule, inject the tubule with dye, then remove the pipette and move the animal board on the stage (with the microscope tube fixed to the base) to bring the various tubule segments into the field. When this is done, however, the tubule at the time the diameter measurements are being made is filled with dye solution not under the same pressure which obtained during the time of intracapsular pressure measurement and the measured diameters would, therefore, not be the same as those obtaining during normal urine flow. It was thus considered necessary to have an arrangement whereby during the period of tubule diameter measurements the dye pipette could be kept in the capsule and the tubule continuously perfused with dye solution at the normal pressure.

The pipettes for collecting the glomerular fluid were drawn from Pyrex

tubing with an outside diameter of about 5 mm. and an inside diameter of about 3.5 mm. The tube was drawn out to a shaft several centimeters long and from 0.5 to 0.8 mm. outside diameter. A tip was then drawn on this shaft, the outside diameter at the extremity being 15 to 20μ. After the pipette was inserted into the capsule the mercury leveling bulb was lowered to produce a negative pressure of 1 or 2 mm. mercury, thus emptying the capsule. The distance from the tip to the junction of the mercury column and the column of preformed glomerular fluid was then immediately measured with the micrometer ocular. During the next ten minutes the leveling bulb was kept at such a level that the capsule wall was just collapsed, the suction pressure being always less than 1 mm. mercury. At the end of ten minutes the distance from the tip to the junction between mercury column and glomerular fluid column was again observed; the difference between this level and the level to which the column of preformed glomerular fluid had come being the length of the column of fluid passing the glomerular membrane during the ten minute collection period. The diameter of the fluid column was obtained by breaking the pipette and measuring its inside diameter, the pipette being so drawn that its inside diameter was uniform throughout the length occupied by the fluid column.

The primary renal tubule of Necturus consists of five morphologically distinguishable segments, the ciliated neck with its nephrostome, proximal convoluted tubule, straight segment, distal convoluted tubule and the junctional tubule passing to a collecting tubule. The reader is referred to Chase's paper (1923) for details of structure. The dye-injected tubule is clearly outlined; it is always possible to distinguish the ciliated neck, the proximal convoluted tubule and the distal segment, consisting of straight segment, distal convoluted tubule and junctional tubule. In some cases the straight segment is sharply outlined between the proximal and distal convoluted tubules, in others the inside diameter of this segment is so nearly that of the distal convolution that their line of demarcation is indistinguishable. It has not been possible to distinguish the junctional from the distal convoluted tubule. If we consider Poisseuille's equation $h = \frac{8v\ \eta l}{t\pi dgr^4}$, where h = head of pressure in centimeters H_2O, v = cc. liquid passing through tube, η = coefficient of viscosity of liquid, l = length of tube in centimeters, t = time in seconds, d = specific gravity of liquid, g = gravity constant, 980, and r = radius of tube in centimeters, we see that we have all the data required for calculating h. $\frac{v}{t}$ or flow in cubic centimeters per second can be obtained from the volume collected in ten minutes, l from the camera lucida drawing and r from the tubule diameter measurements. The specific gravity of the glomerular fluid, d, is taken

as unity, while the coefficient of viscosity, η, is taken as that of water. The pressure head h, is calculated for each segment and the sum taken as the pressure head required to drive the fluid through the tubule at the observed rate. This is then compared with the observed intracapsular pressure. Six technically satisfactory experiments have been performed; the protocols of all are given.

1. October 13. Male. Intracapsular pressure 2.7 cm. H_2O. Preformed fluid came up into the straight shaft of collecting pipette 32 spaces from tip (1 space = 38.4μ). In 10 minutes end of column was 84 spaces from tip, i.e., column of 52 spaces or 2.0 mm. in 10 minutes. Inside pipette diameter (uniform) = 25.4 spaces (1 space = 20.8μ) or 0.528 mm., making $l\pi r^2 = 0.44$ cu. mm. during 10 minutes or 7.3×10^{-7} cc. per sec. $= \frac{v}{t}$. Dye pipette reinserted and dye run in under pressure of 2.9 cm. H_2O.

	Length mm.	Diameter spaces	μ
Ciliated neck	1.17	1.4	22
Prox. tubule	8.6	4.0	64
Distal segment	9.6	2.2	35

Taking formula

$$h = \frac{v8\eta l}{t\pi dgr^4} = \frac{vl}{tr^4} \times 2.6 \times 10^{-5}$$

since

$$\frac{8 \times 0.01}{\pi \times 1 \times 980} = 2.6 \times 10^{-5},$$

we get

$$h_1 \text{ (ciliated neck)} = \frac{7.3 \times 10^{-7} \times .117}{(0.0011)^4} \times 2.6 \times 10^{-5} = 1.5 \text{ cm.}$$

$$h_2 \text{ (prox. tubule)} = \frac{7.3 \times 10^{-7} \times 0.86}{(0.0032)^4} \times 2.6 \times 10^{-5} = 0.16 \text{ cm.}$$

$$h_3 \text{ (distal segment)} = \frac{7.3 \times 10^{-7} \times 0.96}{(0.00175)^4} \times 2.6 \times 10^{-5} = 1.9 \text{ cm.}$$

Total = 3.6 cm. Bladder urine 6.2 mgm. P per 100 cc.

2. October 22. Male. Intracapsular pressure 4.2 to 4.4 cm. H_2O for about 2 minutes. Collected 0.30 cu. mm. in 10 minutes or 5.0×10^{-7} cc. per sec. $= \frac{v}{t}$.

	Length mm.	Diameter spaces	μ
Ciliated neck	1.2	1.3	21
Prox. tubule	9.3	2.9	46
Distal segment	11.3	2.0	32

$$h_1 \text{ (ciliated neck)} = \frac{5.0 \times 10^{-7} \times 0.12}{(0.00105)^4} \times 2.6 \times 10^{-5} = 1.28 \text{ cm.}$$

$$h_2 \text{ (prox. tubule)} = \frac{5.0 \times 10^{-7} \times 0.93}{(0.0023)^4} \times 2.6 \times 10^{-5} = 0.43 \text{ cm.}$$

$$h_3 \text{ (distal segment)} = \frac{5.0 \times 10^{-7} \times 1.13}{(0.0016)^4} \times 2.6 \times 10^{-5} = 2.5 \text{ cm.}$$

Total = 4.2 cm.

3. October 24. Female. Intracapsular pressure 2.4 to 2.6 cm. H_2O for 2 minutes. Collected 0.40 cu. mm. fluid in 10 minutes or 6.7×10^{-7} cc. per sec. $= \frac{v}{t}$.

	Length mm.	Diameter spaces	μ
Ciliated neck	0.7	1.2	19
Prox. tubule	7.3	3.1	50
Distal segment	10.6	2.3	37

$$h_1 \text{ (ciliated neck)} = \frac{6.7 \times 10^{-7} \times 0.07}{(0.00095)^4} \times 2.6 \times 10^{-5} = 1.5 \text{ cm.}$$

$$h_2 \text{ (prox. tubule)} = \frac{6.7 \times 10^{-7} \times 0.73}{(0.0025)^4} \times 2.6 \times 10^{-5} = 0.33 \text{ cm.}$$

$$h_3 \text{ (distal segment)} = \frac{6.7 \times 10^{-7} \times 1.06}{(0.00185)^4} \times 2.6 \times 10^{-5} = 1.58 \text{ cm.}$$

Total = 3.4 cm. Bladder urine 2.0 mgm. P per 100 cc.

4. November 7. Female. Intracapsular pressure 1.6 to 1.7 cm. H_2O for 2 minutes. Collected 0.27 cu. mm. fluid in 10 minutes or 4.5×10^{-7} cc. per sec. $= \frac{v}{t}$. Straight segment between proximal and distal convoluted tubules distinctly outlined in this and fifth experiments. Micrometer scale with 1 division = 8μ used in this and following experiments.

	Length mm.	Diameter spaces	μ
Ciliated neck	1.0	2.5	20
Prox. tubule	9.2	7.0	56
Str. segment	0.8	3.6	29
Distal segment	8.0	4.7	38

$$h_1 \text{ (ciliated neck)} = \frac{4.5 \times 10^{-7} \times 0.1}{(0.001)^4} \times 2.6 \times 10^{-5} = 1.17 \text{ cm.}$$

$$h_2 \text{ (prox. tubule)} = \frac{4.5 \times 10^{-7} \times 0.92}{(0.0028)^4} \times 2.6 \times 10^{-5} = 0.18 \text{ cm.}$$

$$h_3 \text{ (str. segment)} = \frac{4.5 \times 10^{-7} \times 0.08}{(0.00145)^4} \times 2.6 \times 10^{-5} = 0.21 \text{ cm.}$$

$$h_4 \text{ (distal segment)} = \frac{4.5 \times 10^{-7} \times 0.8}{(0.0019)^4} \times 2.6 \times 10^{-5} = 0.72 \text{ cm.}$$

Total = 2.3 cm. Bladder urine 4.8 mgm. P per 100 cc.

5. November 14. Female. Intracapsular pressure 3.5 to 3.8 cm. H_2O for 2 minutes except for brief drop (about 20 sec.) to 3.0 cm. Collected 0.35 cu. mm. fluid in 10 minutes or 5.8×10^{-7} cc. per sec. $= \frac{v}{t}$.

	Length mm.	Diameter spaces	μ
Ciliated neck	0.83	2.8	22
Prox. tubule	9.1	7.6	61
Str. segment	0.67	3.8	30
Distal segment	13.3	4.8	38

$$h_1 \text{ (ciliated neck)} = \frac{5.8 \times 10^{-7} \times 0.083}{(0.0011)^4} \times 2.6 \times 10^{-5} = 0.86 \text{ cm.}$$

$$h_2 \text{ (prox. tubule)} = \frac{5.8 \times 10^{-7} \times 0.91}{(0.0031)^4} \times 2.6 \times 10^{-5} = 0.15 \text{ cm.}$$

$$h_2 \text{ (str. segment)} = \frac{5.8 \times 10^{-7} \times 0.067}{(0.0015)^4} \times 2.6 \times 10^{-5} = 0.2 \text{ cm.}$$

$$h_4 \text{ (distal segment)} = \frac{5.8 \times 10^{-7} \times 1.33}{(0.0019)^4} \times 2.6 \times 10^{-5} = 1.54 \text{ cm.}$$

Total = 2.8 cm.

6. December 7. Male. Intracapsular pressure 2.4 cm. H_2O, constant for 2 minutes. Collected 0.24 cu. mm. fluid in 10 minutes or 4.0×10^{-7} cc. per sec. $= \frac{v}{l}$.

	Length *mm.*	Diameter spaces	μ
Ciliated neck	1.4	3.0	24
Prox. tubule	10.4	8.5	68
Distal segment	8.2	4.4	35

$$h_1 \text{ (ciliated neck)} = \frac{4.0 \times 10^{-7} \times 0.14}{(0.0012)^4} \times 2.6 \times 10^{-5} = 0.7 \text{ cm.}$$

$$h_2 \text{ (prox. tubule)} = \frac{4.0 \times 10^{-7} \times 1.04}{(0.0034)^4} \times 2.6 \times 10^{-5} = 0.08 \text{ cm.}$$

$$h_3 \text{ (distal segment)} = \frac{4.0 \times 10^{-7} \times 0.82}{(0.00175)^4} \times 2.6 \times 10^{-5} = 0.9 \text{ cm.}$$

Total = 1.7 cm. Bladder urine 9.8 mgm. P per 100 cc.

Drawings of the tubules used in these six experiments are given in figure 1. In the drawing of the tubule of the first experiment the measured widths of the various segments have been inked in as nearly as possible according to scale. The inside diameters are represented, the variations in wall thickness not being shown. The lengths are quite accurately to scale. The other five drawings are simply tracings of the single-line camera lucida drawings reduced one-half, i.e., the figures as they appear are fifteen times natural size. In these drawings no attempt has been made to represent the relative widths of the various segments; their limits are designated by transverse lines. The nephrostomes are not shown, since the dye did not enter them at the low pressures under which it was run in.

It is seen that in every case the observed head of intracapsular pressure agrees reasonably well with the calculated head, being sometimes greater, sometimes less, the extremes showing the observed pressure in one experiment (4) 28 per cent less than the calculated and in another (6) 41 per cent higher. These variations represent approximately the probable errors of the methods. The conclusion appears justified that practically no water is reabsorbed by the tubules, certainly not more than 30 or 40 per cent of the amount eliminated by the glomeruli.

A consideration of the various factors which may introduce errors into these comparisons may be taken up here. The question may first be raised as to how accurately the volume of fluid collected in ten minutes represents the volume which would have entered the glomerular capsule during the same period without the capsular puncture. Two possible sources of

error work against each other here. The first is that some of the fluid may escape the pipette and pass down the tubule, but with a ready exit

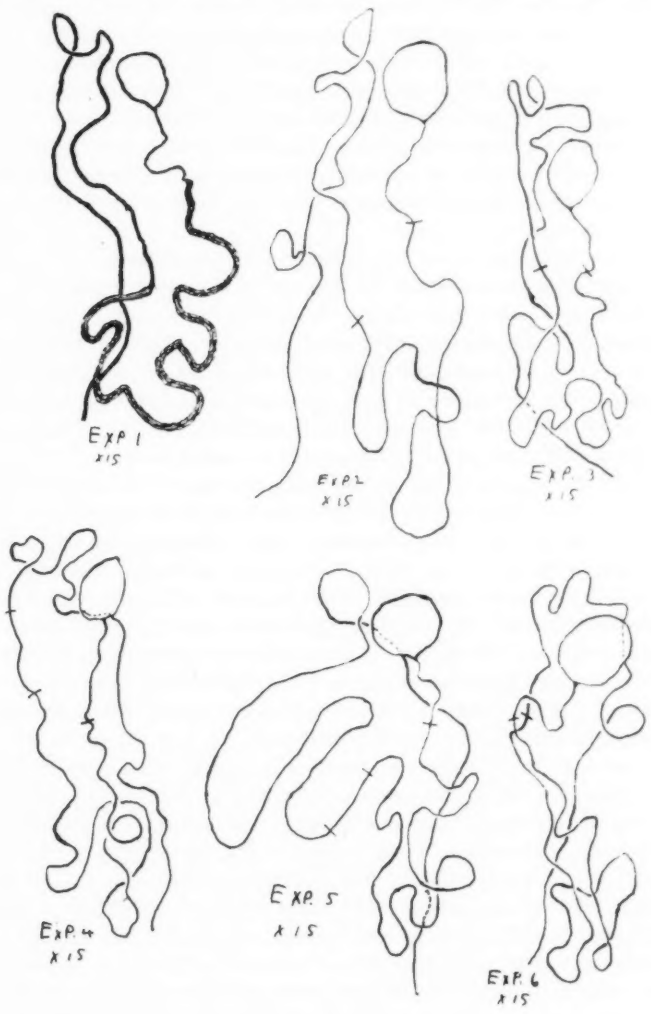

Fig. 1

into the pipette under slight negative pressure it is quite improbable that more than a small fraction of the fluid will escape. The other factor is

that the release of the positive intracapsular pressure by the capsular puncture will tend to increase the rate of formation of glomerular fluid since the effective filtering pressure head is now increased. Just how nearly these opposing factors cancel each other to give a collected volume equal to that which would have entered the unpunctured capsule it is impossible to say. I believe the two errors are of about the same magnitude, although it is probable that the increase due to the lowered intracapsular pressure is somewhat greater than the amount escaping down the tubule. In other words, the collected volume is probably somewhat greater (by not more than 15 to 20 per cent) than the volume which would have entered the unpunctured capsule.

The second question is that of errors in tubular measurement. Errors in diameter measurements will, of course, be the most important. The precautions against overdistention of the tubules have already been mentioned. The question may be raised as to how accurately the measured width of the dye column inside the tubule represents the tubule's inside diameter. There are two theoretically possible sources of error, each of which would make the measured width greater than the actual lumen; first, the possibility of absorption of the dye by the tubule wall next to the lumen, and second, the possibility of magnification of the dye column by refraction by the tubule wall. To avoid the first of these either a colloidal dye, trypan blue, or a 1 to 4 dilution of water soluble India ink was used as the injecting fluid. Early in this work considerable time was spent in the effort to get the inside diameters of the injected tubules by making serial sections of the tissues frozen or fixed by various chemical fixatives and imbedded by various techniques. The conclusion drawn from this part of the work was that direct observation of the width of the dye column with the kidney *in situ* afforded a more accurate measure of tubule lumen than did the cross sections and that the magnification was negligible, since the diameters observed directly were practically always smaller than those in the sections. In the first three experiments an ocular micrometer scale with the lines 0.1 mm. apart was used, in the last three a scale with the lines 0.05 mm. apart, one scale division being equivalent to 16 and 8μ, respectively. Since one can estimate to fifths of a scale division it is seen that the error of measurement in the first three experiments may be about 3μ and in the last three less than 2μ. A calculation will show that an error of 2μ will introduce an error of about 40 per cent in the calculated head of pressure for the ciliated neck, of about 15 per cent for the proximal tubules and of about 25 per cent for the distal segment. Since it is hardly likely that the errors of measurement of all the segments would ever be in the same direction it appears that the calculated pressure head of the entire tubule will not be in error by more than 20 to 30 per cent due to errors in tubule diameter measurements. A given tubule segment does not, of

course, have an absolutely uniform lumen throughout its length; the variations are significant, however, only in the wide proximal tubule where they have relatively little effect on the total calculated pressure head and even here an average can be arrived at with reasonable accuracy. The tubule lengths can be measured quite accurately. The only significant error is that due to foreshortening when the tubule dips out of the horizontal plane and since the primary tubules nowhere depart very far from a given horizontal plane, this error is not great. The lengths of the various segments were measured on the camera lucida drawings by a tracer wheel of 20 mm. circumference, with divisions 2 mm. apart. The drawing was 30 times the size of the tubule.

The error introduced by taking the specific gravity of the glomerular fluid as unity is negligible. The coefficient of viscosity of water is 0.01 at 20°, that of 1 per cent NaCl is the same. Since the glomerular fluid is protein-free its coefficient of viscosity has been taken as 0.01; the slight variations due to variations in room temperature can introduce errors of only a few per cent. The measurements of intracapsular pressure are quite accurate, the error is less than 1 mm. H_2O.

Another factor which may be supposed to cause a discrepancy between the calculated and observed pressure heads in the capsule is the activity of the cilia in the ciliated neck and in the straight segment. The calculation of the pressure head required to drive a liquid at a given rate through a tube of given dimensions depends, of course, upon the condition that no further propulsive force is applied to the liquid, whereas in the tubule the cilia exert a propulsive force away from the capsule and thus might be expected to lower the pressure in the capsule. At the same time, however, the cilia of the nephrostome are forcing fluid from the outside into the ciliated neck, so that below the junction of nephrostome with ciliated neck there is a greater volume of fluid passing through the tubule than is leaving the capsule. This will have the effect of raising intracapsular pressure. I have attempted to evaluate these opposing factors.

The first question to be answered is, "To what extent does the propulsive action of the cilia in the ciliated neck lower the intracapsular pressure." To answer this question two pipettes were inserted into a single glomerular capsule. One had outside and inside diameters at the tip of about 20 and 14μ, respectively, and into its distal portion was drawn a 20 per cent solution of urethane in either Janus green solution or India ink dilution. The other end of this pipette was connected with a leveling bulb according to the previously described method for determining intracapsular pressure. The other pipette was drawn from Pyrex capillary tubing with outside and inside diameters of about 5 mm. and 1.3 mm., respectively, and had at the tip outside and inside diameters of 16 to 18μ and 4 to 4.5μ, respectively. This second pipette was filled with water and connected with a leveling

bulb filled with water. The resistance to the flow of liquid through such a pipette is about 10 to 15 times that of a single tubule. The leveling bulb of the second pipette was raised to such a height that the intracapsular pressure resulting from the flow of water from the bulb was somewhat higher than the previously existing intracapsular pressure. This provided an arrangement whereby the tubule could be perfused at a constant rate of flow; since most of the resistance in the pipette-tubule system was in the pipette, variations in the resistance of the tubule would produce negligible changes in the rate of flow. Therefore, changes in the resistance of the tubule, as brought about by changes in ciliary activity, would manifest themselves by changes in intracapsular pressure.[1] The intracapsular pressure produced by the water perfusion was now determined while the cilia were active; only an insignificant amount of the urethane-dye solution leaves the pipette during this procedure. The leveling bulb of the high resistance perfusion pipette was next lowered and the bulb of the urethane-dye pipette raised until the solution ran down the ciliated neck and entered the proximal tubule; the latter was then occluded by pressing on it with a fine glass rod in order to prevent the narcotic from passing through the entire tubule where it might conceivably alter the diameter and thus introduce another variable into the tubule resistance. After two minutes the leveling bulb of the urethane-dye pipette was lowered and as much as possible of the solution sucked back into the pipette; the leveling bulb of the perfusion pipette was again placed at its original height, the rod occluding the tubule was removed and the constant flow of water through the tubule thus reëstablished. After the urethane-dye contents of the capsule had been sufficiently washed out the intracapsular pressure was again determined.[2] It is evident that if the cilia are exerting a significant propulsive action the intracapsular pressure will rise if their activity is abolished while the rate of flow remains constant. This was found to be the case in both experiments, the protocols of which are given here.

[1] It had been established that two minutes' exposure to the urethane-dye or urethane-ink solution would completely abolish ciliary activity in the frog's pharynx and that this was not reëstablished by five minutes' washing with Ringer's solution.

[2] The most difficult feature of these procedures is the second determination of the intracapsular pressure. Two successful experiments were performed out of many attempts. The failures were due to the fact that as a result of the urethane-dye injection to abolish ciliary activity the capsule was so stained that it could not be washed clear enough by the subsequent water perfusion to permit a visualization of the dye-containing pipette tip sufficiently distinct for the second intracapsular pressure determination. Janus green was found more satisfactory than trypan blue; the latter dye is apparently adsorbed on the capsular wall. Janus green stains the capillary tuft a deep blue, but the capsule wall, while somewhat stained, is not rendered opaque, at least not in every case.

1. November 24. Male. Intracapsular pressure with leveling bulb of perfusing pipette 45 cm. above tip was originally 31 mm. H_2O, after ciliary activity had been abolished by urethane, 35 mm. H_2O, a rise of 4 mm. Water soluble India ink diluted 1 to 2 used as injected fluid, which also, of course, contained 20 per cent urethane.

2. December 1. Female. Intracapsular pressure with leveling bulb 40 cm. above tip was originally 17 mm. H_2O, after urethane was 22 mm. Janus green used to color injecting fluid.

It is seen that while the cilia exert an appreciable influence, the intracapsular pressure which would obtain without the cilia is lowered by only 12 to 23 per cent by their activity, at the pressures investigated.

The second of the factors due to ciliary activity is that due to the cilia of the nephrostome. These are forcing fluid into the tubule, thereby raising the intracapsular pressure. An approximation of the extent of this effect may be reached in the following way. If the leveling bulb attached to a dye-containing pipette in a capsule is raised until the dye is forced out to the open end of the nephrostome, against its ciliary action, and then lowered to the level of intracapsular pressure, the dye is rapidly swept through the nephrostome into the ciliated neck. A length of 1 mm. of nephrostome with an inside diameter of about 20μ will be cleared of dye in about 5 seconds; the time required varies rather widely on either side of this average from one nephrostome to the next, but successive determinations on the same nephrostome give practically constant times. Taking the figure of 5 seconds as the average it is seen that a column of liquid 1 mm. long and of 20μ diameter enters the tubule from the nephrostome in 5 seconds, i.e., 0.038 cu. mm. in 10 minutes. Since an average of about 0.35 cu. mm. of fluid enters the tubule from the capsule in 10 minutes it is seen that about 11 per cent more is added by the nephrostome, which would raise the intracapsular pressure by something less than 11 per cent. It is thus seen that the resultant of the two factors due to ciliary activity is a decrease in intracapsular pressure of probably 10 to 15 per cent.

A comparison of observed with calculated intracapsular pressures is justifiable only on the condition that the rate at which the glomerulus is eliminating fluid is substantially the same during the collection period and at the time of making the intracapsular pressure determinations. I have never observed in Necturus any evidence of alternation of glomerular capillary circulation which might cause sudden changes in the rate of glomerular fluid elimination over a short period of time; furthermore, intracapsular pressure has been followed for as long as ten minutes on several occasions and found to show no significant changes.

To restate the original problem, if we know the rate of flow at a given temperature of a liquid of known specific gravity and viscosity through a tube of known dimensions we can calculate the pressure head necessary to maintain this flow. The calculation is, of course, made on the condition

that all the liquid goes all the way through the tube. In so far as the calculated pressure head agrees with the observed the conclusion is justified that there is no exchange of liquid between the wall and lumen of the tube. The various respects in which the problem in the renal tubule differs from that in an inanimate tube have been considered. Thus the factor $\frac{v}{t}$ or rate of flow, as determined by the rate of collection of fluid in a pipette, is less than the normal rate of flow down the tubule by whatever extent the fluid escapes down the tubule during the collection and by the extent to which the nephrostome adds fluid to the tubule; it is greater than the normal flow by the extent to which the lowering of intracapsular pressure by the capsular puncture increases filtration. The effect which a given $\frac{v}{t}$ will have on the pressure head is lowered by the extent to which the cilia in the tubule exert a propulsive influence on the liquid. If we consider all the factors which will tend to make the intracapsular pressure differ from that calculated by Poisseuille's equation we see that they largely cancel each other. On the basis of the reasonably close agreement between the observed and calculated pressures reported in these experiments the conclusion appears justified that there is no significant exchange of water between the wall and lumen of the tubule.

We may consider briefly the application of this conclusion to the general theory of renal activity. Since it is known that glucose and chloride are reabsorbed it appears that a reabsorption of water is not essential to an absorption of solute. The most valuable feature of the establishment of the fact that there is no water reabsorption in these tubules, or that if there is it is compensated by an output of water by them, is that it provides a way out of the old dilemma as to whether the urinary concentration of certain substances is due to a secretion of these substances or to a reabsorption of water. We frequently see the urinary concentration of certain constituents of mammalian urine 100 times that of the plasma. This demands, on the pure filtration-reabsorption theory, that 99 liters of water be reabsorbed in the tubules for each liter of urine reaching the bladder. Necturus urine is not concentrated enough with respect to any urinary constituent to demand such a tremendous reabsorptive capacity of the tubules but its phosphate concentration may be several times that of the glomerular fluid. In two experiments reported in a recent paper by Schmitt and White (1928) where urine was collected during a period of glomerular fluid collection the urine phosphate concentration was between three and four and between four and five times that of the glomerular fluid phosphate concentration. In those experiments there was no way to decide whether this concentration was effected by water reabsorption or by tubular secretion of phosphate but if no water is reabsorbed it is clear that

secretion is proved. In the experiments reported in the present paper no urine collections during the course of the experiments were made but the urine in the bladder when the animal was opened was taken when available. These urines had 6.2, 2.0, 4.8 and 9.8 mgm. P per 100 cc. Since it has been established by Schmitt and White that the glomerular fluid P concentration in Necturus is constantly less than 2 mgm. per 100 cc., with an average of 1.6, it is seen that the urine P concentration in the present experiments is from slightly over one to six times that of the glomerular fluid concentration and since no significant amount of water is being reabsorbed the tubules must be secreting phosphate. The rate of tubular phosphate secretion is variable, at times practically none being added, at others enough to make the urine concentration several times that of the glomerular fluid.

No nitrogen figures were obtained in the present experiments, the only available figures are those of two cases reported by White and Schmitt (1926) in one of which there were 7.4 and 12 mgm. urea N per 100 cc. of blood and urine, respectively, and in the other 7.6 and 11 mgm. total non-protein nitrogen per 100 cc. of plasma and urine, respectively. It thus appears that there is only a slight tubular secretion of urea. Necturus urine is constantly very low in chloride, about 0.02 per cent expressed as NaCl; water reabsorption is not essential to chloride reabsorption. The absence of Henle's loop in this kidney together with the demonstration of a failure of water reabsorption afford a confirmation of the inference drawn from comparative renal physiology that the loop is the chief site of water reabsorption.

Brodie's calculations (1914) of the pressure head existing in the dog's glomerular capsule may be mentioned here. He determined the rate of urine flow from a dog's kidney and measured the diameters of the various tubular segments of the kidney fixed in 10 per cent formalin. The number of glomeruli in the kidney was determined and the lengths of the various segments taken from measurements by Peter (1907). The rate of flow from one glomerulus and the dimensions of the tubule being thus known he calculated the pressure head to be 83 mm. Hg in one experiment and 50.3 mm. Hg in another.[3] The possibilities of error in work of this kind are, I believe, considerably greater than in the work reported in the present paper. In the first place, the lumens of the tubular segments reported by

[3] It is obvious that Brodie's figure of 8μ for the diameter of the collecting tubule in his second experiment (p. 577) is an error, for with that figure this segment alone would have a pressure head of 107 mm. Hg. On the assumption that the error is typographical and that 18μ was meant I have calculated the total pressure head as 50.3 mm. Hg. There must be an arithmetical error in Brodie's calculations which give 74.1 mm. Hg as the pressure head in the second experiment; this figure is not obtained either with 8 or 18μ as the collecting tubule diameter.

Brodie are almost certainly too small. His tissues were fixed in formalin; the sections of Necturus kidney in which I found the measured cross sections of the tubules to be as great as or greater than the inside diameters as observed *in vivo* were fixed in Bouin's or Lavdowsky's fluids which produce less shrinkage. Furthermore, differences in the structure of kidneys of different species might produce widely differing extents of shrinkage by one and the same fixative; the fact that the inside diameters of Necturus tubules are not diminished by the fixatives used does not mean that the inside diameters of dog tubules are not diminished by formalin. It may be pointed out that the inside tubule diameters reported by Brodie are less than the average seen in dog's kidneys fixed in Zenker's or Bouin's fluids. It may also be noted that the percentage error in calculated pressure head introduced by a given error of tubular diameter measurement is very much less in working with the relatively wide Necturus tubule than with the narrow tubule of the dog. In the second place, Brodie's figures of 125,000 and 142,000 glomeruli per kidney are less than half of the figure of 300,000 reported by Peter; the uncertainty as to the number of glomeruli in the kidney does not, of course, enter into my work If we recalculate Brodie's experiments taking the diameters of the various segments as 4μ greater than his figures we get 22.3 instead of 82.6 mm. Hg in the first experiment and 14.6 instead of 50.3 in the second. If we use Peter's figure of 300,000 glomeruli in a dog's kidney instead of those given by Brodie the capsular pressure heads are further reduced to 10.5 mm. Hg in the first experiment and 6.1 mm. Hg in the second, which are not at all improbable intracapsular pressures in the dog. Brodie, of course, had no figures for observed intracapsular pressure. Speculations concerning water reabsorption in the dog's tubule based on one's guess as to how high the dog's intracapsular may rise would lead us too far afield; furthermore, the data are not adequate even for speculating since no figures on the relative concentrations in urine and plasma of any of the urinary constituents are given.

SUMMARY

The head of pressure in the glomerular capsules of Necturus calculated by Poisseuille's equation was found to agree substantially with the observed intracapsular pressure. The conclusion is drawn that there is no significant exchange of water between the tubule wall and lumen.

The propulsive action of the cilia lowers the intracapsular pressure by only 12 to 23 per cent.

Since the phosphate concentration of the bladder urine may be several times that of the glomerular fluid it is concluded that the tubule cells secrete phosphate.

Reabsorption of water is not essential to a reabsorption of glucose or chloride.

The loop of Henle is probably the chief site of water reabsorption in the mammalian tubule.

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SOME MEASUREMENTS OF CILIARY ACTIVITY

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Received for publication December 20, 1928

In the preceding paper (White, 1929) were reported two measurements of the propulsive action, as evidenced by their effect on lowering intracapsular pressure, of the cilia in the ciliated neck of Necturus renal tubules during a period of normal rate of flow of liquid through the tubules. The present paper reports some measurements of maximum pressures which ciliary activity can maintain.

In the first set of experiments a dye-containing pipette connected with a leveling bulb was inserted into a glomerular capsule and enough dye run in to identify the corresponding proximal tubule. The capsule was then torn wide open, the pipette, with the tip toward the capsule, inserted into the proximal tubule a short distance below its junction with the ciliated neck, and the tubule occluded distal to the pipette by pressure with a fine glass rod. Ringer's solution was kept dripping over the kidney surface. The height to which the pressure in the proximal tubule now rises is a measure of the pressure difference which the cilia can build up by forcing liquid down into the tubule. This intratubular pressure is measured by noting the height of the leveling bulb at which the dye in the pipette tip is in equilibrium with the intratubular liquid. In six such experiments, done on four animals, the tubule pressures were 5.2, 4.0, 4.6, 5.4, 4.8 and 5.7 cm. H_2O. The pressure rises gradually, reaching the maximum in from 1 to 2 minutes and then remaining constant; at the time of maximum pressure the tubule above the occlusion is widely distended. It is thus seen that the pressure difference which the cilia can build up is much greater than the extent (4 or 5 mm. H_2O) to which they lower the intracapsular pressure with a normal rate of flow through the tubule.

It may be pointed out here that this demonstration answers an objection which has been raised (Pütter, 1926) against regarding the fluid collected by a pipette in a capsule as being purely capsular fluid; it has been objected that the fluid may be in part that which has been drawn back from the tubule. This objection appears reasonable in the case of the experiments of Wearn and Richards (1924), against which it was directed. They employed suction pressures of 20 to 30 mm. Hg in drawing the capsular

fluid into the pipette, an unnecessarily high degree of suction. If, however, the fluid is drawn into the pipette with a negative pressure of not more than 1 mm. Hg it is evident that it will not be contaminated by tubular fluid, since the cilia of the ciliated neck can resist a pressure of several millimeters Hg. And since the findings of Wearn and Richards on the capsular fluid of frogs have been found to hold in Necturus, where the fluid was collected under a negative pressure of not more than 1 mm. Hg, it appears probable that even with the suction pressures which they employed there was no significant contamination of capsular by tubular fluid.

Another set of experiments was carried out to determine the maximum pressure against which the cilia of the nephrostome can prevent liquid from passing out, in the reverse of the normal direction, from the ciliated neck to the mouth of the nephrostome. In these experiments a dye-containing pipette connected with a leveling bulb was inserted into a capsule and dye run in to identify the proximal tubule, which was then occluded by a glass rod a short distance below its junction with the ciliated neck. The leveling bulb was then raised until dye was seen passing out from the ciliated neck through the nephrostome and the pressure at which this occurred was observed. After this observation, in some of the experiments the same determination which was carried out in the first set of experiments was also obtained, i.e., after tearing open the capsule, removing the pipette from the capsule and removing the tubular occlusion until the tubule had been washed clear, the pipette was inserted into the proximal tubule, which was again occluded and the intratubular pressure determined. Also, in some of these experiments the intracapsular pressure was determined before and after occluding the proximal tubule.

1. December 10. Female. Dye came out of nephrostome when intracapsular pressure was 8.5 to 9 cm. H_2O, was slowly swept back into ciliated neck when bulb was lowered to 8 cm.

2. December 14. Male. Intracapsular pressure before proximal tubule occlusion was 3.4 cm. H_2O, after occlusion rose to 8.6 cm. H_2O. Dye moved out of nephrostome with intracapsular pressure of 10.2 cm. H_2O, was slowly swept back into ciliated neck when bulb was lowered to 9.7 cm.

Another capsule in the same animal showed intracapsular pressure of 3.8 cm. H_2O before tubule occlusion, 9 cm. afterwards. Dye moved out of nephrostome when bulb was raised to give intracapsular pressure of 10.8 cm. H_2O, slowly swept back when bulb was lowered to 10.3 cm.

3. December 14. Female. Intracapsular pressure 2.2 cm. H_2O before tubular occlusion, 7.8 cm. afterwards. Dye came out of nephrostome with intracapsular pressure of 8.5 to 9 cm. H_2O, came back at 8 cm. Next tore capsule, removed tubular occlusion and let tubule wash out, then put pipette into proximal tubule and again occluded tubule distal to pipette. Tubular pressure rose to 4.4 cm. H_2O.

In another capsule intracapsular pressure was 3.0 cm. H_2O before tubular occlusion and 8.5 cm. afterwards. Dye left nephrostome at intracapsular pressure of 11.0 to 11.5 cm. H_2O, came back at 10.5 cm. Next tore capsule, removed tubular occlusion and let tubule wash out, then put pipette into proximal tubule and occluded tubule distal to pipette. Tubular pressure rose to 5.3 cm. H_2O.

It is seen that the nephrostome cilia can prevent an outward passage of liquid against a considerable pressure. It may be pointed out that the maximum pressure against which the nephrostome cilia can prevent the outward passage of liquid is even somewhat greater than the pressure indicated by the height of the leveling bulb, since, due to the activity of the cilia in that portion of the ciliated neck between the capsule and the origin of the nephrostome, the pressure in the ciliated neck at the origin of the nephrostome is somewhat greater than the intracapsular pressure determined by the height of the leveling bulb.

The intracapsular pressures with the tubule unoccluded are of course those pressures due to the passage of fluid into the capsule across the glomerular membrane. An interesting point arises in regard to the interpretation of the intracapsular pressure after the tubule is occluded. At first thought one might consider this procedure analogous to the determination of maximum ureteral pressure with the ureter occluded, the pressure being due to the entrance of fluid into the capsule across the glomerular membrane. It must be remembered, however, that with the tubule occluded, the nephrostome cilia will sweep liquid into the tubule until the intratubular pressure reaches the maximum height against which the nephrostome cilia are able to move liquid. The intracapsular pressure will always, of course, be somewhat less than this, due to the activity of the cilia in that portion of the ciliated neck between the capsule and the origin of the nephrostome.

It thus appears that the maximum intracapsular pressure with the tubule occluded cannot be taken as a measure of the maximum pressure against which liquid can enter the capsule through the glomerular membrane for the former can never exceed the maximum pressure against which the nephrostome cilia can prevent the escape of liquid into the body cavity; it will, in fact, be somewhat less than this, due to the activity of the cilia in that portion of the ciliated neck between the capsule and the origin of the nephrostome. If the glomerulus can still pass liquid into the capsule against the maximum intracapsular pressure which the nephrostome cilia can build up with the tubule occluded, the liquid thus entering the capsule will escape into the body cavity through the nephrostome, thus preventing any rise of intracapsular pressure above that which the nephrostome cilia can build up. The present experiments do not permit us to decide whether or not this may happen; a crucial experiment to determine the maximum pressure against which the glomerulus can eliminate liquid would necessitate occlusion of the nephrostome as well as of the tubule and up to the present time this has not been technically possible.

The above observations do not exclude the possibility that in the capsules from which pass tubules not bearing nephrostomes (and these are in the great majority, only the primary tubules having nephrostomes) the pressure due to the passage of liquid across the glomerular membrane

may rise, with the tubule occluded, higher than the pressure which the nephrostome cilia were observed to exert. These secondary capsules in Necturus are not accessible for manipulation.

Such a consideration does not, of course, enter into an interpretation of maximal ureter pressure with the mammalian kidney; there (if ureteral contractions, the valve-like action of the papillae of the kidney pelvis, possible osmotic effects between tubule contents and blood, and perhaps other factors are allowed for) it is truly a measure of the maximum pressure against which the glomeruli can eliminate fluid.

SUMMARY

The cilia in the first segment of the renal tubule of Necturus can, with the capsule torn and the proximal tubule occluded, raise the pressure in the proximal tubule proximal to the occlusion to 4.0 to 5.7 cm. H_2O. It is pointed out that this demonstration proves that capsular fluid withdrawn under suction of not more than 1 to 2 mm. Hg cannot be contaminated with tubular fluid.

The nephrostome cilia can exert pressures of 8 to 11 cm. H_2O. The complication which this introduces into an interpretation of the maximum intracapsular pressure with the tubule occluded as a measure of the maximum pressure against which the glomeruli can eliminate fluid is discussed.

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THE EFFECT OF PROLONGED GENERAL ANESTHESIA AND OF DECEREBRATION ON THE LACTIC ACID AND GLYCOGEN CONTENT OF MAMMALIAN SKELETAL MUSCLE

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Received for publication December 21, 1928

When a cat is decerebrated, the muscles (especially the extensors) become hypertonic. This hypertonicity is said to be different from ordinary steady contraction in that it does not exhibit fatigue. Electromyograms of muscles after decerebration show oscillations which may be due to somatic motor impulses (1), (2). Metabolism studies have been made on animals in decerebrate rigidity, and results have been variable. Roaf (3) found that in decerebrate cats the O_2 intake and CO_2 output were no greater in the state of decerebrate rigidity than in an atonic condition, i.e., after curare or after cutting the nerves to the limbs. According to Bayliss (4), Lovatt Evans found the metabolism to be less after curare than in decerebrate rigidity. Bayliss (5) studied the heat production in decerebrate rigidity and reported that there was a certain amount of extra heat produced, which varied in magnitude with the degree of tonic contraction, although it was less than would have been produced in an artificial tetanus of a similar magnitude. Dusser de Barenne and Burger (6) determined the gas exchange in decerebrate animals and said that it was generally 10 to 12 per cent greater than in the absence of rigidity. In one case, the O_2 intake was 25 per cent greater.

Our work was undertaken to study the lactic acid metabolism of decerebrate rigidity in adult cats. If decerebrate rigidity depends upon a special tonic mechanism, it might be that the lactic acid content of the blood and muscle would show no change. On the other hand, if it involves the ordinary somatic motor mechanism, it might be expected that there would be lactic acid changes both in the muscles and the blood during rigidity. The excitation of a muscle by motor nerve impulses always causes an increase of lactate. At least there is, so far as we are aware, no experimental exception to this premise. Hill, Long and Lupton (7) and Ronzoni and Wallen-Lawrence (8) have shown by analysis of human blood taken immediately after exercise that there is a free diffusion of lactic acid from muscles into the blood stream. Cori and Cori (9) state that epinephrin injections, violent exercise, strychnine and insulin convulsions, asphyxia, decerebra-

tion and a variety of other conditions lead to an escape of lactate from the muscle. They (10) found a marked increase of blood lactic acid in decerebrate cats. In decerebrate dogs, Himwich, Koskoff and Nahum (11) have demonstrated a marked increase in blood lactic acid, the main source of which was the muscles.

It should be remembered that in the observations to be recorded here the blood supply to the muscles was intact and that we were studying the lactic acid and glycogen content under conditions of an adequate oxygen supply. There are so many factors which enter into this phase of metabolism of decerebrate rigidity that it makes interpretation of results difficult. Among these factors are the general physiological state of the animal, the condition of the respiratory mechanism, the blood presssure and vascular tone, the influence of the anesthetic used in preparing the animal, the changes in body temperature, and the possible interference with normal activity of functions controlled by the brain stem due to the decerebration. We have endeavored to control as many of these factors as possible and believe that our data are indicative of the mechanism involved.

PROCEDURE. For determinations of lactic acid in muscle, we have used one gastrocnemius in which the nerve supply had been removed by section of the sciatic nerve in the thigh, either before or after decerebration, and the other gastrocnemius with its nerve supply intact. Thus we have worked with one tonic gastrocnemius and the opposite atonic one. In order to insure an equal blood supply to both muscles, in some of the experiments the lumbar sympathetic trunk on each side (ganglia L 4–L 7) was removed through a median abdominal incision, at least a week prior to decerebration. By this operation, the vasoconstrictor impulses to the muscles of the limbs should have been removed and thus section of the sciatic nerve on one side should not cause a marked increase of blood supply to the atonic muscle. To be sure, section of the sciatic might cause a stimulation of posterior root vasodilator fibers in the nerve but one would expect this to be transitory. In regard to the lactic acid content of the muscles, the normal and sympathectomized cats showed essentially the same values.

The cats were decerebrated by the transection of the brain stem at the level of the superior colliculi. In most of the experiments, they were under ether for about one-half hour. The common carotids were ligated and care was exercised to prevent excessive hemorrhage. In two experiments, the cats were decerebrated during local anesthesia (1 per cent novocaine) in order to prevent any rise in lactic acid which might be attributed to a specific effect of ether. There were no marked respiratory disturbances following the decerebrations and with very few exceptions the cats were in excellent condition. Rectal temperatures were recorded and the body temperature was maintained at a normal level by an electric heating pad. Since the respirations were nearly normal and the rigidity was maintained

throughout the experiments, the general physiological condition of the animal seemed not to have been particularly disturbed. After from one to six hours following decerebration, the gastrocnemii on the two sides were dissected free from surrounding tissues, but the blood supply and the origins and insertions were left intact. The muscles were allowed to rest about ten minutes after the skin incision had been closed. Then the skin incision was opened and the muscles were frozen *in situ*, with the technique described by Davenport and Davenport (12), using a mixture of CO_2 snow and ethyl chloride. The atonic muscle was usually frozen first since the freezing of a denervated muscle would cause no reflex responses. However, the reverse order was sometimes followed as a matter of control. The frozen muscles were sliced across the fibers into sections 0.1 to 0.2 mm. thick, and immediately put into ice-cold 5 per cent trichloracetic acid. Lactic acid determinations were made with the method of Friedemann, Cotonio and Shaffer (13) with modifications in apparatus described by Davenport and Davenport (12). Glycogen was determined by the alkali method of Pflüger followed by the Shaffer-Hartmann procedure for sugar after its hydrolysis by acid. The blood samples in most cases were taken from venous blood but in some instances heart's blood was used.

Results. The resting lactic acid value for guinea pig and rat muscle has been reported previously (12) as about 15 mgm. per cent. This value was obtained with amytal anesthesia but is similar to those obtained for resting muscles of frogs, and also for the lactic acid content of the blood of resting human beings. Hefter and Judelowitsch (14) found a lactic acid level in resting human beings of 9 to 11 mgm. per cent. In one experiment (not included in the tables) with a cat under amytal anesthesia, the lactic acid content of the gastrocnemii was 7 and 10 mgm. per cent respectively and for blood from a femoral vein 6 mgm. per cent. In another cat a sample of heart's blood, obtained under local anesthesia, in the presence of a moderate amount of struggling, contained 16 mgm. per cent lactic acid. In spinal cats, Irving (15) found lactic acid values in muscle as low as 6 and 8 mgm. per cent. It would seem that the low values with amytal are not to be ascribed to an interference with carbohydrate metabolism but to the flaccidity and resting state of the muscle brought about by the central nervous action of amytal.

In a series of twelve cats decerebrated under ether, eight showed a higher lactic acid content on the tonic side than on the atonic. In most of these cats, the lactate content of venous blood was higher than any reasonable resting level. The values obtained in the muscles were also higher than any probable resting value. In some of the atonic muscles, the lactic acid content was likewise higher than resting. This would be expected if lactate from the blood stream diffused into them. These high values might be thought to be caused by raising the level of lactic acid by ether

used during decerebration. Ronzoni, Koechig and Eaton (16) showed that ether anesthesia in dogs causes an acidosis of the lactic acid type which disappears three to five hours after discontinuing the anesthesia. In three experiments (recorded here, table 2), cats were put under deep ether anesthesia. After one-half hour, blood samples were taken and, in each case, one muscle was frozen and the leg amputated. The anesthesia was continued in two cats for three hours and in the third four hours after the first muscle was taken. A fall in both blood and muscle lactate was observed at the end of anesthesia. The glycogen was essentially the same in two and dropped in a third one. It is evident that in the presence of continued ether anesthesia in the cat that the lactic acid level tends to fall rather than to rise.

In experiment XIX (table 2), ether was administered for 45 minutes and the cat was then given amytal. The muscle and blood in the presence of ether show much higher values than under amytal two and one-half hours later. We believe that there is a relationship of the different lactate levels observed during ether and amytal anesthesia to the difference in the tonicity of the muscles. It was found impracticable to keep cats so deeply anesthetized with ether for several hours that muscle tone was abolished. Even during ether anesthesia of moderate depth, the animals showed considerable stiffness of the limbs—a tonicity which resembled but was less pronounced than decerebrate rigidity. Amytal anesthesia produced a rag-like flaccidity and was associated with a lower lactate level than ether anesthesia. In two experiments (XXIV and XXVI, table 2), cats were decerebrated and then given amytal. The lactic acid content of both blood and muscles was then found to be low. It would seem reasonable to conclude that the decrease should be attributed to the reduction of muscular activity.

We have endeavored to eliminate the initial effect of ether by allowing intervals as long as six hours to elapse after decerebration and discontinuance of ether anesthesia before the muscle was removed. As a further control, two experiments were made wherein decerebration was done under local anesthesia (1 per cent novocaine) in the complete absence of ether. These cats (XXII and XXIII, table 1) possessed a high degree of rigidity, the blood lactate was high in both, and the tonic muscles had a higher lactate content than the atonic in both cases. It seems safe to conclude that the rise in lactic acid cannot be attributed solely to the effect of ether.

Glycogen determinations were made in nine experiments (table 1). In two, the glycogen was equal (within experimental error) on the tonic and atonic sides. In a third it was higher on the tonic side. In the remaining six, there was a higher glycogen content on the atonic side.

Long (17) has reported a decrease of about 20 per cent in glycogen content of resting muscles of cats under amytal anesthesia. We have

TABLE 1

Lactic acid and glycogen in decerebrate rigidity

One gastrocnemius was denervated by section of the sciatic nerve. Sympathectomy was done by bilateral excision of lumbar sympathetic trunk with L4–L7 ganglia.

NUMBER*	LACTIC ACID GASTROCNEMIUS	GLYCOGEN	LACTIC ACID BLOOD	SOURCE OF BLOOD	INTERVAL BETWEEN DECEREBRATION AND FREEZING	REMARKS
	mgm. per cent	*mgm. per cent*	*mgm. per cent*		*hours*	
N II	T† 37		36	Femoral veins	1½	
	A 20		40			
S III	T 26		27	Femoral veins	1½	Tonic muscle frozen first
	A 18		34			
S IV	T 33		25	Femoral veins	1½	Tonic muscle frozen first
	A 26		22			
S V	T 35		32	Femoral veins	1½	Tonic muscle frozen first
	A 46		38			
S IX	T 24		11 dupli-	Heart	5	
	A 20		9 cates			
S X	T 26	290	19	Heart	6	
	A 29	360				
N XI	T. 14	574	9	Heart	6	Rigidity not marked
	A 12	589	12			
N XII	T 41	471	11	Heart	6	
	A 23	496				
N XIII	T 36	690	24	Heart	6	
	A 36	635				
N XIV	T 44	500			¾	Respirations abnormal and condition of animal poor
	A 49	500				
N V	T 37	595			5½	
	A 33	645				
S XXI	T 45	815	43	Ext. jugular vein	5	
	A 27	810				
S XXII	T 43	1,070	45	Duplicates from inf. vena cava	5	Decerebrated under local anesthesia
	A 33	1,120	44			
S XXIII	T 41	750	53	Duplicates inf. vena cava	5	Decerebrated under local anesthesia
	A 37	825	58			

* N = normal cat, S = sympathectomized cat.

† T = tonic gastrocnemius, A = atonic gastrocnemius.

TABLE 2

Lactic acid and glycogen content of muscles (gastrocnemii) and lactic acid content of blood during general anesthesia

	ANIMAL NUMBER	SAMPLE NUMBER	DURATION OF ANESTHESIA	LACTIC ACID	GLYCOGEN	REMARKS
			hours	*mgm. per 100 gm.*	*mgm. per 100 gm.*	
Cats	N XVI	M 1	¾	60	1,000	
		M 2	3¼	25	1,000	
	N XVII	M 3	¾	49	755	Normal animals Sciatic nerves intact Ether anesthesia throughout
		M 4	3¼	39	620	
		B 3	¾	54		
		B 4	3¼	36		
	N XVIII	M 5	¾	105	670	
		M 6	3¼	39	685	
		B 5	¾	98		
		B 6	3¼	38		
	N XIX	M 7	¾	28	770	Same as above except amytal substituted for ether after first ¾ hour
		M 8	3¼	5	730	
		B 7	¾	42		
		B 8	3¼	8		
	S XXIV	M 9	2	13	725	Decerebrated under ether. B 9 taken 1 hour after beginning anesthesia, then amytal given and B-10, M-9, and M-10 taken 1 hour later
		M 10	2	13	695	
		B 9	1	20		
		M 10	2	19		
	S XXVI	M 11	4	11	830	Sciatic to M-11 cut under ether before decerebration. Amytal given 1½ hours after decerebration and all samples taken 2½ hours later
		M 12	4	12	800	
		B 11	4	5		
Guinea pigs	NA	M 13	½		1,030	No shivering. Temp. fell 4°C.
		M 14	4		1,070	
	NB	M 15	½		1,080	Marked shivering. Temp. fell 3°C.
		M 16	4		1,140	
Rats	NC	M 17	½		680	Light anesthesia. Slight fall in temp. No shivering
		M 18	4		640	
	ND	M 19	½		540	Deep anesthesia. Marked fall in temp. No shivering
		M 20	4		570	

Amytal throughout

repeated this work in principle on the gastrocnemii of two guinea pigs and two rats (table 2). In these experiments, the animals were given amytal and one-half hour later one gastrocnemius was frozen and the leg amputated. Three and one-half hours later, the other gastrocnemius was frozen. The variations are within the limits of experimental error and within the expected variation in similar muscles of the same animal. We believe, therefore, that a sweeping conclusion that the glycogen content of unstimulated mammalian muscle decreases during general anesthesia is unjustifiable.

Discussion. These observations show that there is a relatively high lactic acid level in the blood during decerebrate rigidity. Since the skeletal muscles of the body are hypertonic after decerebration and since the production of lactic acid is associated chiefly with muscular contraction, it follows that the lactic acid level may be attributed to the increased activity of the skeletal musculature. We have made no direct measurement of the tension developed in decerebrate rigidity, hence cannot estimate the amount of lactic acid which would be expected according to the formula developed by Hill and Meyerhof. We need precise information in regard to tension-length relationships before we can make a comparison between the tetanic contractions of skeletal muscles and the contractions of decerebrate rigidity. Higher lactic acid in tonic muscles in most experiments furnishes evidence of lactic acid production by decerebrate rigidity, but no explanation for the few atonic muscles which contained as much or more lactic acid than the corresponding tonic ones can be given.

This increased lactic acid production might account for the results obtained by Evans and Dusser de Barenne and Burger and for the increase in heat production reported by Bayliss. Whether or not the ordinary contractile mechanism will account for all of the muscle activity in decerebrate rigidity remains an unsolved question. However, we do know that action potentials are present in the electromyograms of decerebrate muscles. There is also good evidence for an increase in metabolism as shown by the greater O_2 intake and CO_2 output, for an increase in heat production and an increase in lactic acid production.

Himwich and co-workers (11) state that in decerebrate dogs, the main source of lactic acid is the muscle while the organ chiefly concerned with its removal is the liver. From the data in table 1, it is evident that the lactate level of venous blood is in most cases higher than in the blood of the heart. It is probable that the difference is due to the activity of the liver in removing the lactic acid.

Our data are not comprehensive enough to draw any conclusions concerning glycogen variations in the tonic and atonic muscles. It may be noteworthy that in six of nine experiments the glycogen was slightly lower in the tonic muscle. One would expect that there would be a greater differ-

ence between the glycogen level in the tonic and atonic muscles if lactic acid is being formed by the tonic ones, but it is possible that, if glycogen is being used, it is replaced as rapidly as it is being broken down. The glycogen content of corresponding muscles from the two sides of the same animal does not vary beyond the experimental error for its determination (18), (17). This fact should be considered in any interpretation of glycogen variations. It is evident from table 2 that, with the exception of cat XVII, there are no variations of sufficient magnitude to be interpreted as showing that ether and amytal cause a decrease in glycogen content of the muscle. In the guinea pig NB (table 2), there was marked shivering under amytal without decrease in glycogen. Long (17) stimulated the sciatic nerve at the rate of 60 per minute for 30 minutes in amytalized cats and found immediately and after 3 hours a marked diminution in glycogen on the stimulated side. In some unpublished observations, we have confirmed this observation. If shivering depends upon glycogen for its source of energy, the glycogen is replaced under amytal.

Ronzoni, Koechig and Eaton (16) showed that, in dogs, the accumulation of lactic acid accounts chiefly for the acidosis of ether anesthesia, that decreased O_2 supply to the tissues does not account for its production and that the source of lactic acid seems to be the muscular tissue. Our observations show an initial increase in lactic acid during the beginning of ether anesthesia but a decrease in both the muscle and blood three to four hours later. The initial increase can be attributed to the muscular activity during the early stages, for marked hypertonicity is often present at that time. Why this level is not maintained in the cat as it is in the dog is not explained by the data available. When all muscular tone is abolished by amytal anesthesia following ether, the level is decreased.

SUMMARY

1. The blood lactic acid level of decerebrate cats is higher than the resting one.

2. In 10 of 14 experiments the lactic acid content of the tonic gastrocnemius was higher than that of the atonic one.

3. The glycogen content of the tonic and atonic muscles was essentially the same. In 6 of 9 experiments it was slightly higher on the atonic side.

4. In cats under ether anesthesia, the lactic acid level is initially high but tends to drop as anesthesia progresses.

5. The glycogen content of the unstimulated gastrocnemii of the rat and guinea pig does not decrease under amytal anesthesia of three and one-half hours.

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STUDIES IN HUMAN PHYSIOLOGY

II. Pulse Rate and Blood Pressure

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Received for publication December 8, 1928

This is a continuation of our report on intra-individual variation of physiological functions, the first and preceding installment of which (1) dealt with weight, temperature and basal metabolism. This paper will present data concerning the systolic blood pressure and pulse rate under basal conditions and standing, and the pulse rate after a standard exercise, together with the cardiovascular rating of Schneider (2) which is derived from these measurements.

The subjects (A. B., C. D., E. F., G. H. and K. L.) and the general plan and routine of the determinations have been described in our preceding report and need not be repeated here. The cardiovascular measurements were made as follows:

During the preliminary rest period which preceded the basal metabolism determination a sphygmomanometer (mercury manometer type) was adjusted to the subject's arm (usually the right). After the subject had been reposing quietly for thirty or forty-five minutes, and just before commencing the metabolism determination, the systolic pressure was determined by the auscultatory method, using the first sound as criterion. As soon as the collection of the expired air was completed and after the subject had been relieved of mouth-piece and nose-clip, but before any movement, the pressure was again determined. And when there was a second collection of the expired air, as was generally the case, the pressure was again determined upon its completion.

The basal pulse rate was counted for one minute at the beginning, middle and toward the end of every ten-minute metabolism determination.

The subject then arose and stood quietly, but not rigidly, for several minutes, usually four or five. At the end of this time the pulse rate and systolic pressure were redetermined until it was apparent that they had reached a steady state. The sphygmomanometer was then detached and the subject performed the standard light exercise, described by Schneider, of stepping five times in fifteen seconds upon a laboratory stool about 18 inches in height. The pulse rate during the first fifteen seconds following

TABLE 1

Statistical constants for the cardio-vascular data

FUNCTION	SUBJECT	NUMBER OF OBSERVATIONS	MAXIMUM AND MINIMUM	MODE	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	AVERAGE DEVIATION FROM THE MONTHLY MEAN; PER CENT OF THE MEAN	AVERAGE DEVIATION FROM THE YEARLY MEAN; PER CENT OF THE MEAN	EXTREME DEVIATIONS FROM THE YEARLY MEANS; PER CENT OF THE MEAN
Basal pulse rate	A. B.	259	56–76	64	65.2 ±0.2	3.89	5.97	3.1	4.3	−13.8; +16.9
	C. D.	243	48–65	53	54.0 ±0.2	3.52	6.53	3.7	4.4	11.1; 20.4
	E. F.	66	54–80	66	66.2 ±0.5	5.49	8.30	5.1	6.1	18.2; 21.2
	G. H.	53	61–83	68	68.9 ±0.5	5.24	7.60	4.4	6.3	10.2; 20.3
	K. L.	161	50–74	61	59.9 ±0.3	6.26	10.45	4.9	6.5	16.7; 23.3
	Av.				62.8	4.88	7.77	4.2	5.5	−14.0; +20.4
Standing pulse rate	A. B.	94	72–96	88	85.2 ±0.4	5.23	6.13	3.1	4.7	−15.3; +12.9
	C. D.	89	60–112	96	86.3 ±0.7	9.36	10.90	5.0	8.5	30.2; 30.2
	E. F.	40	68–106	72	81.4 ±1.1	10.50	12.90	6.9	7.1	16.0; 30.9
	G. H.	37	80–104	88	92.0 ±0.6	5.27	6.63	4.0	4.5	13.0; 13.0
	K. L.	44	80–108	96	96.1 ±0.7	6.63	6.90	3.9	5.2	16.7; 12.5
	Av.				88.2	7.40	8.69	4.6	6.0	−18.2; +19.9
Pulse rate after exercise	A. B.	94	84–120	104	102.0 ±0.5	7.78	7.62	3.6	5.8	−17.6; +17.6
	C. D.	89	68–124	92	96.6 ±0.6	8.41	8.70	3.8	6.6	29.9; 27.8
	E. F.	40	76–116	104	98.4 ±1.0	8.94	9.09	5.2	7.0	22.4; 18.4
	G. H.	37	107–132	116 120	116.2 ±0.5	5.00	4.30	2.5	3.2	7.8; 13.8
	K. L.	44	94–136	120	117.6 ±0.9	8.49	7.22	3.8	5.7	11.9; 15.3
	Av.				106.2	7.72	7.39	3.8	5.7	−17.9; +18.6

Basal systolic blood pressure	A. B.	101	82–109	98 99	97.3 ±0.4	5.51	5.66	2.1	4.4	−15.5; +12.4
	C. D.	98	93–115	108	106.1 ±0.3	5.06	4.76	2.4	3.4	12.3; 8.5
	E. F.	50	95–108	98	101.2 ±0.3	3.29	3.26	1.9	2.7	5.9; 6.9
	G. H.	46	103–125	112	113.9 ±0.5	5.18	4.55	2.5	3.6	9.4; 9.4
	K. L.	50	89–111	96	99.4 ±0.5	5.05	5.08	1.7	4.3	10.1; 12.1
	Av.				103.6	4.82	4.66	2.1	3.7	−10.6; +9.9
Standing systolic blood pressure	A. B.	94	82–112	99 100	97.5 ±0.4	6.04	6.20	2.6	5.1	−16.3; +14.3
	C. D.	89	95–122	105	106.4 ±0.4	5.76	5.33	2.5	4.3	10.4; 15.1
	E. F.	40	85–122	99 104 105	103.0 ±0.8	7.42	7.21	3.6	5.2	17.5; 18.5
	G. H.	37	89–121	106	109.0 ±0.7	6.04	5.67	3.3	3.9	18.4; 11.0
	K. L.	44	89–109	94 95 98	96.8 ±0.5	4.84	5.00	2.5	4.2	8.3; 12.4
	Av.				102.5	6.02	5.88	2.9	4.5	−14.2; +14.3

completion of the exercise was recorded and the counting was continued until there was return to the standing normal, or until the end of the second minute after the exercise in case it had not returned within that time.

In our calculations we have used the average of the three one-minute counts, made during each ten-minute metabolism determination, as the basal pulse rate. When there were two metabolism determinations, as was usually the case, each average has been regarded as a duplicate pulse determination. There was usually no difficulty about determining the standing pulse; but in case consecutive, fifteen-second counts showed much variation, the one of greatest frequency was used because the pulse rate after exercise, which was to be compared with it, had to be based on a single, fifteen-second count.

As has been said above, several readings were made of both the basal and standing systolic pressures in the course of any one determination. These readings, however, were not recorded in such a way as to be useful in determining the variability of duplicate determinations; instead, we have recorded either the average of all the readings made at one time, or, in case one value predominated, it was taken as typical.

All of the data obtained in the course of this entire work were intended to supply standards for normal physiological functions and to that end no determinations were made when there was any obvious illness. It was hoped in addition, however, that an intensive study of this kind might reveal some physiological basis for those variations in physical fitness which at present have no more exact or elegant description than that of "feeling fine" or "feeling rotten." As a first approximation to this end it was felt desirable to have as objective a record as possible of the variations in physical efficiency. For this purpose we chose Schneider's cardiovascular rating (2) from among the number that have been proposed because it fitted in most readily with the other routine of the determinations.

The chief interest in this work, as in that covered in our preceding report, was in connection with the possibility of seasonal periodicity. Since, however, we have been unable to find anything in the literature concerning the intra-individual variation to be expected in these functions, a statistical analysis of these data may be of value. We shall also include observations as to the effect of sleep on the basal pulse rate and of menstruation on the different functions studied.

I. STATISTICS. In table 1 are given the usual statistical constants that are useful in establishing the characteristics of such material as this. Considering the relative variability of the different functions it will be seen that the blood pressure (systolic) either basal or standing, has a lower variability, whether measured absolutely by the standard deviation, or

relatively to the respective means by the coefficient of variation and the average deviations, than the basal or standing pulse rates; i.e., the blood pressure for either condition is more constant than the corresponding heart rate.

In addition, the standing values, both of pressure and heart rate, show a greater variability on the average than the basal values. On the other hand there is not nearly so great a difference between the variability of the standing pulse and the pulse after exercise as there is between the basal and standing conditions; relative to the means the variability is actually less, the average coefficients of variation being 8.69 and 7.39 for standing and after exercise, respectively; also the average deviations from the monthly and yearly means show a greater variability for the standing condition; only the standard deviation has a slightly higher value, 7.72, after exercise than for the standing condition, 7.40. This is probably merely confirmation of what has already been pointed out (3), viz., standing still imposes a greater strain on the organism than is usually recognized.

The variability in all of these functions increases with the time interval over which the observations extend, i.e., the average deviation from the monthly means is consistently less than that from the yearly means. It is unfortunate that we are unable in every case to compare with these the variability of duplicate determinations. As was mentioned above, our data were recorded in such a way as to make this possible only in the case of the basal pulse rate. For this function we had, for nearly every morning's work, two average values, both of which were based on the three counts made during each of two consecutive metabolism determinations. Considering these as duplicate pulse determinations we have the following as the average deviation of such duplicate determinations from their means:

	per cent
A. B.	1.7
C. D.	1.4
E. F.	2.7
G. H.	1.2
K. L.	2.0
Average	1.8

If this, 1.8 per cent, is compared with the average deviations from the monthly and yearly means which are 4.2 and 5.5 per cent, respectively, it is seen that the greatest variability in such a series of observations is imposed by bona fide, day-to-day variations; and that these, in turn, are superimposed upon variations of longer duration. This is similar to the conclusion reached in the preceding report regarding the basal oxygen consumption.

In the preceding report it was also shown that every phase of the metabolism was more variable in the women than in the men. This relationship does not extend to the cardio-vascular findings as is shown by the following averages:

	AVERAGE COEFFICIENT OF VARIATION		AVERAGE STANDARD DEVIATION	
	Men	Women	Men	Women
Basal pulse rate	6.25	8.78	3.71	5.66
Standing pulse rate	8.51	8.81	7.29	7.47
Pulse rate after exercise	8.16	6.87	8.09	7.48
Basal blood pressure	5.21	4.29	5.28	4.51
Standing blood pressure	5.76	5.96	5.90	6.10
Average	6.78	6.94	6.05	6.24

Both absolutely and relative to their respective means, the women are slightly more variable on the average than the men. As can be seen, however, this difference is not consistently shown in all of the functions.

In addition to the statistical constants that measure variability, we have also been interested in the degree of correlation existing between the several factors of the circulation themselves; and, in addition, that between the circulation and such other functions as the basal metabolism and temperature, for which there is already known to be some inter-individual correlation.

The degree of correlation between the different measurables of the circulation is shown in the following tabulation (it must be remembered that it is the correlation between the intra-individual variations of these functions that is being measured):

Coefficients of correlation between the

STANDING PULSE RATE	PULSE RATE AFTER EXERCISE	BASAL BLOOD PRESSURE	STANDING BLOOD PRESSURE	SUBJECT
(1) Basal pulse rate and				
+0.20 ±0.13	+0.29 ±0.13	−0.43 ±0.11	−0.10 ±0.14	A. B.
+0.30 ±0.13	+0.34 ±0.12	+0.11 ±0.14	−0.24 ±0.13	C. D.
+0.41 ±0.16	+0.27 ±0.18	−0.28 ±0.18	−0.61 ±0.13	E. F.
+0.72 ±0.19	+0.15 ±0.18	+0.33 ±0.17	+0.60 ±0.12	G. H.
+0.81 ±0.07	+0.62 ±0.12	+0.85 ±0.05	+0.52 ±0.14	K. L.
+0.36	+0.33	+0.12	+0.03	Av.

STANDING PULSE RATE	PULSE RATE AFTER EXERCISE	BASIL BLOOD PRESSURE	STANDING BLOOD PRESSURE	SUBJECT
	(2) Standing pulse rate and			
	+0.75 ±0.06	+0.31 ±0.12	+0.10 ±0.14	A. B.
	+0.84 ±0.04	+0.35 ±0.12	−0.27 ±0.13	C. D.
	+0.84 ±0.06	−0.39 ±0.17	−0.35 ±0.18	E. F.
	−0.21 ±0.18	+0.10 ±0.19	−0.29 ±0.17	G. H.
	+0.81 ±0.07	+0.47 ±0.15	+0.19 ±0.18	K. L.
	+0.61.....	..+0.37.....	..−0.12.....	.....Av.
	(3) Pulse rate after exercise and			
		+0.30 ±0.13	+0.20 ±0.13	A. B.
		+0.28 ±0.13	−0.02 ±0.14	C. D.
		−0.13 ±0.19	−0.23 ±0.19	E. F.
		+0.19 ±0.18	+0.25 ±0.18	G. H.
		+0.26 ±0.18	+0.02 ±0.20	K. L.
		..+0 18.....	..+0.04.....	.....Av.
	(4) Basal blood pressure and			
			+0.69 ±0.07	A. B.
			+0.52 ±0.10	C. D.
			+0.44 ±0.16	E. F.
			+0.53 ±0.14	G. H.
			+0.88 ±0.05	K. L.
			+0.61.....	.....Av.

From this it follows that there is really significant correlation only between *1*, the standing pulse rate and the pulse rate after exercise; and *2*, between the basal and standing blood pressure. The first might certainly have been expected since standing is but one form of exercise; and perhaps the second relationship is just as logical in spite of the apparent disparity between the basal and standing condition, since if a high pressure is required to meet the needs of the body at rest it could hardly be supposed that a diminished pressure would be adequate to fulfill the increased demands of exercise.

The correlation between pulse rate and metabolism under basal conditions has long been known and is constantly referred to in the literature (4), (5); but we have never been able to find a quantitative statement of the intra-individual relationship comparable to that which we have for the inter-individual correlation (6). Below, we give the constants derived from our own data for the basal pulse rate as well as the basal blood pressure; in both cases the correlation with the protein metabolism has been computed separately for the years 1925 and 1926, because during

the former the nitrogen determinations were made on 24-hour urines and in the latter year on the urines excreted during the metabolism periods (1).

Coefficients of correlation between the

TOTAL OXYGEN	TOTAL CARBON DIOXID	PROTEIN OXYGEN		SUBJECT
		1925	1926	
(1) Basal pulse rate and				
+0.37 ±0.04	+0.24 ±0.04	−0.04 ±0.09	+0.10 ±0.07	A. B.
+0.23 ±0.04	+0.30 ±0.04	+0.10 ±0.10	+0.12 ±0.07	C. D.
+0.47 ±0.06	+0.15 ±0.08	+0.08 ±0.10		E. F.
+0.45 ±0.07	+0.46 ±0.07	+0.10 ±0.10		G. H.
+0.52 ±0.04	+0.44 ±0.04		+0.23 ±0.07	K. L.
+0.41	+0.32	+0.06	+0.15	Av.
(2) Basal systolic blood pressure and				
−0.18 ±0.07	−0.15 ±0.07	−0.34 ±0.09	−0.20 ±0.09	A. B
+0.28 ±0.06	+0.10 ±0.07	+0.25 ±0.09	−0.06 ±0.09	C. D.
0.00	+0.06 ±0.09	−0.22 ±0.09		E. F.
−0.17 ±0.10	−0.06 ±0.10	−0.08 ±0.10		G. H.
+0.70 ±0.05	+0.49 ±0.07		+0.33 ±0.08	K. L.
+0.13	+0.09	−0.10	+0.02	Av.

It may be said at once that there is no significant correlation between the blood pressure and metabolism, nor between the pulse rate and protein metabolism. The correlation between the pulse rate and total oxygen consumption or carbon dioxid production, however, is consistently positive and significant in value; indeed, the coefficients are greater for these intra-individual variations than those found by Harris and Benedict (6) inter-individually. This is particularly true of the women, for whom they found a very low inter-individual relationship. Incidentally it may be mentioned that the inter-individual correlation between basal pulse and oxygen consumption (1) for the three women of this group is practically a straight line function, as may be seen from the following tabulation:

SUBJECT	AVERAGE BASAL	
	Pulse rate	Oxygen
		cc. per minute
Women:		
K. L.	60	181
E. F.	66	186
G. H.	69	188
Men:		
A. B.	65	177
C. D.	54	200

With these subjects it is the men who are obviously anomalous, insofar as the inter-individual correlation is concerned.

The inter-individual correlation between pulse rate and body (oral) temperature was found by Whiting (7) to be $+0.288 \pm 0.020$ in 927 pairs of observations on 500 individuals; and Lyon (8) found a similar value, $+0.263$, for the average intra-individual correlation in six non-febrile subjects on each of whom were made from 35 to 59 observations; in the latter series the individual values ranged from the insignificant figure of -0.021 to the rather significant value of $+0.534$. Although it is not specifically stated, these observations presumably apply to the sitting position; it is interesting, therefore, to compare them with our data which were obtained under basal conditions.

SUBJECT	COEFFICIENT OF CORRELATION PULSE RATE AND ORAL TEMPERATURE BASAL CONDITIONS
A. B.	$+0.11 \pm 0.08$
C. D.	$+0.32 \pm 0.05$
E. F.	$+0.04 \pm 0.10$
G. H.	$+0.13 \pm 0.10$
K. L.	$+0.02 \pm 0.08$
Average	$+0.12$

Although all of the correlations are positive only one of them, C. D., is of any importance; the average reflects the prevailing impression that there is no significant relationship between the basal pulse rate and oral temperature, intra-individually.

Inter-individually, the relation between basal pulse rate and oral temperature is shown in the following table where the average values for each of these subjects are arranged in order of increasing body temperature (taken from 1):

SUBJECT	AVERAGE BASAL	
	Pulse rate	Oral temperature
C. D.	54	36.2
E. F.	66	36.4
A. B.	65	36.5
G. H.	69	36.5
K. L.	60	36.9

If K. L. is taken out the remaining members of the group show a fair conformity to the rule of increased pulse rate with increased temperature;

such exceptions as K. L. need not be unexpected, however, when the low average value of the correlation is remembered.

III. THE EFFECT OF SLEEP ON THE BASAL PULSE RATE. In our preceding report evidence was given that going to sleep during a ten-minute metabolism determination had, at most, a negligible effect on the oxygen consumption. The effect on the basal pulse rate may be derived from the same observations, which, it may be restated (1) were as follows: A. B. went to sleep during the first determination of twenty duplicate metabolism periods; there are ten such instances in the case of C. D.; and, for comparison, an equal number of duplicate periods from the same times of the year, but during both of which the subjects were wide awake, have been taken at random for controls. The average figures are as follows:

SUBJECT	AVERAGE BASAL PULSE RATE OF PAIRS OF DUPLICATE DETERMINATIONS		
	First	Second	Difference
A. B. (W. 20)*	63.8	63.6	+0.2
A. B. (S. 20)	62.8	64.6	−1.8
C. D. (W. 10)	52.4	52.1	+0.3
C. D. (S. 10)	50.6	52.2	−1.6

* W., awake; S., asleep; 20 and 10 are the number of observations upon which the averages are based.

That is, when the subjects were awake during both of two duplicate determinations the pulse rate was higher for the first than the second by 0.2 (A. B.) and 0.3 (C. D.); on the other hand when they slept during the first period the average pulse rate for this period becomes lower than that for the succeeding "awake" period by 1.8 (A. B.) and 1.6 (C. D.). Or, in round numbers, sleep reduced the pulse rate on an average of two beats per minute.

It will not be amiss to repeat here the qualification which must attach to this result. This does not pretend to define the effect of deep sleep on the pulse rate. During the ten minutes at their disposal these subjects could not do more than doze or sleep very lightly. The result has significance only in connection with such sleep as may be obtained during a ten-minute metabolism determination.

III. THE EFFECT OF MENSTRUATION. It was shown in a previous section that, on the whole, there is no greater variability among the women than among the men of this group in regard to these cardio-vascular functions. This, in itself, would seem to reduce the probability of any pronounced menstrual disturbance of the pulse and blood pressure. And it may be said at once that the evidence for a menstrual effect here is much less

TABLE 2

The effect of menstruation on the cardio-vascular functions

Averages of the observations made during the menstrual periods and during the first, second, third and fourth weeks, respectively, of the intermenstrual period. The numbers in parentheses are the number of observations on which the average is based.

FUNCTION	SUBJECT	MENSTRUAL PERIOD	INTER-MENSTRUAL PERIOD			
			First week	Second week	Third week	Fourth week and longer
Systolic blood pressure: basal	E. F.	101 (6)	101 (14)	100 (9)	100 (10)	103 (11)
	G. H.	113 (7)	113 (11)	114 (9)	113 (10)	116 (9)
	K. L.	99 (4)	100 (12)	101 (12)	101 (12)	98 (10)
	Av.	105 (17)	104 (37)	104 (30)	104 (32)	105 (30)
Systolic blood pressure: standing	E. F.	101 (5)	103 (13)	105 (9)	100 (6)	105 (7)
	G. H.	112 (6)	110 (10)	107 (6)	109 (9)	108 (6)
	K. L.	98 (4)	98 (11)	99 (10)	96 (10)	97 (9)
	Av.	104 (15)	103 (34)	103 (25)	101 (25)	102 (22)
Pulse rate: basal	E. F.	67 (6)	65 (14)	69 (9)	68 (10)	65 (11)
	G. H.	67 (7)	67 (11)	71 (9)	71 (10)	71 (9)
	K. L.	58 (6)	60 (20)	61 (19)	61 (18)	61 (18)
	Av.	64 (19)	63 (45)	66 (37)	66 (38)	65 (38)
Pulse rate: standing	E. F.	82 (5)	78 (13)	86 (9)	85 (6)	78 (7)
	G. H	91 (6)	90 (10)	93 (6)	93 (9)	94 (6)
	K. L.	95 (4)	97 (11)	97 (10)	96 (10)	95 (9)
	Av.	89 (15)	88 (34)	92 (25)	92 (25)	89 (22)
Pulse rate: after exercise	E. F.	100 (5)	95 (13)	100 (9)	101 (6)	99 (7)
	G. H.	117 (6)	114 (10)	112 (6)	117 (9)	121 (6)
	K. L.	114 (4)	118 (11)	118 (10)	117 (9)	120 (9)
	Av.	110 (15)	108 (34)	110 (25)	113 (24)	114 (22)
Cardio-vascular rating	E. F.	11 (5)	13 (13)	12 (9)	11 (6)	12 (7)
	G. H.	8 (6)	9 (10)	8 (6)	8 (9)	6 (6)
	K. L.	8 (4)	7 (11)	8 (10)	7 (9)	8 (9)
	Av.	9 (15)	10 (34)	9 (25)	8 (24)	9 (22)

conclusive than it was in connection with the metabolism, where it showed itself even in the measures of gross variability. This inconclusiveness is due, however, not so much to lack of variation in each woman subject

throughout the menstrual cycle as to lack of agreement in the details of the variations. Nevertheless there is usually sufficient agreement on some essential point, such as placing the highest or lowest values during the menstrual or intermenstrual period, to justify a brief analysis of the data presented in table 2 in order to determine how much importance to attach to the grand averages which are shown graphically in figure 1.

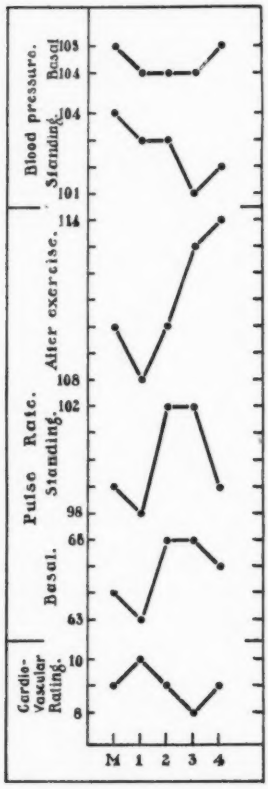

Fig. 1. The effect of menstruation on the cardio-vascular functions; grand averages from table 2; *M*, *1*, *2*, *3*, *4* are, respectively, the menstrual period and the first, second, third and fourth weeks of the inter-menstrual period.

Basal blood pressure. The curve of figure 1 indicates that there is little or no significant change in this function throughout the cycle and this is the only conclusion that is justified by the divergent individual data.

Standing blood pressure. All of the subjects agree in showing a depression toward the end of the inter-menstrual period so that in this respect the average curve of figure 1 is valid. It is not so certain, however, that the pressure is highest during the period itself; with two of the subjects, E. F. and K. L., it is higher during the first part of the inter-menstrual period.

Pulse rate. The three pulse rates, basal, standing and after exercise, are so similar in their variations that they may all be treated together. There is no doubt that the average curves of figure 1 (grand averages of table 2) are correct in showing an inter-menstrual maximum. The post-menstrual decline which these curves show is not so easy to evaluate; it is shown by only one of the subjects, E. F. for the basal pulse; but is quite pronounced for both E. F. and G. H., for the pulse rate standing and after exercise; K. L., on the other hand, shows no indication of it for any of the pulse rates.

Cardio-vascular rating. The grand averages (fig. 1 and table 2) represent quite accurately the results shown by E. F. and G. H., because the third subject is entirely negative.

IV. Seasonal variation. The individual monthly averages are given in table 3 and the grand averages are shown graphically in figure 2.

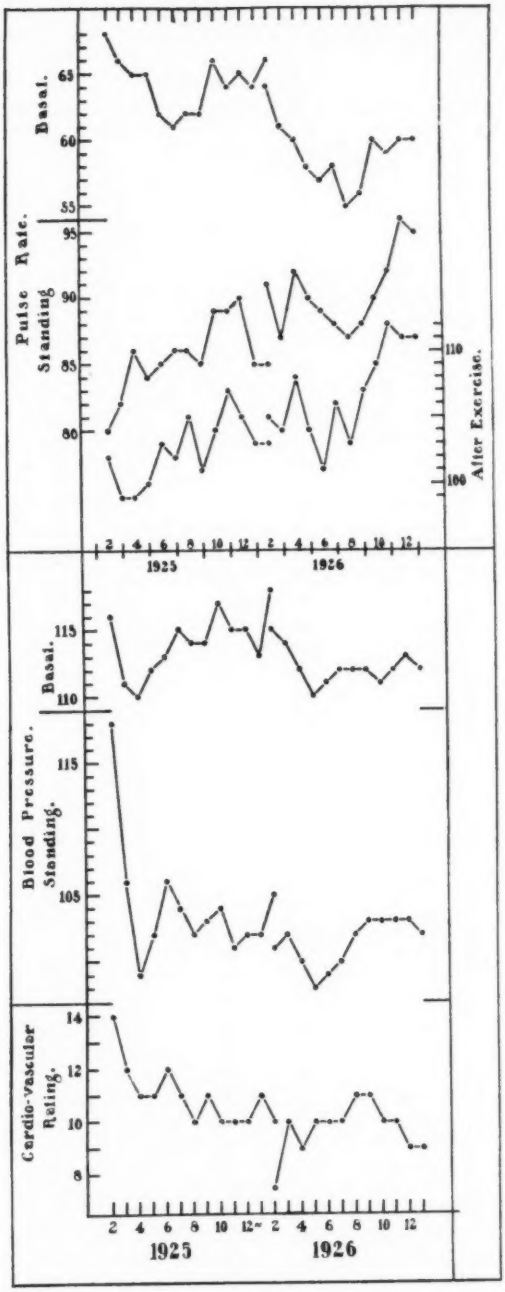

Fig. 2. Seasonal variation; grand averages computed from the data of table 3.

TABLE 3

Monthly averages for the cardio-vascular measurements

SUBJECT	YEAR	MONTH	SYSTOLIC BLOOD PRESSURE		PULSE RATE			SCHNEIDER'S CARDIO-VASCULAR RATING
			Basal	Standing	Basal	Standing	After exercise	
A. B.	1925	February	105	112	69	88	105	14
		March	90	100	70	83	96	14
		April	89	91	69	86	97	13
		May	93	96	67	84	98	13
		June	98	104	66	77	98	14
		July	100	101	62	80	95	13
		August	90	90	61	79	95	13
		September	93	98	64	78	95	15
		October	96	97	68	86	104	12
		November	92	91	70	86	109	11
		December	93	92	70	88	113	9
	1926	January	88	88	68	88	102	11
		February	98	97	68	88	104	10
		March	100	93	64	82	96	11
		April	99	95	65	88	102	10
		May	99	95	63	88	100	11
		June	102	96	62	87	100	10
		July	106	100	64	87	103	10
		August	102	99	61	85	104	12
		September	101	102	63	84	105	12
		October	101	103	66	90	107	11
		November	102	103	65	91	110	10
		December	100	104	64	89	107	11
	1927	January	100	101	64	92	108	10
C. D.	1925	February	103	120	66	70	85	17
		March	106	111	56	74	88	14
		April	105	104	52	80	84	13
		May	104	109	52	73	82	14
		June	106	108	52	76	92	13
		July	105	103	53	88	97	11
		August	107	104	54	92	102	10
		September	107	102	54	89	96	10
		October	110	106	59	99	105	8
		November	105	103	57	93	100	10
		December	105	100	56	95	98	9
	1926	January	107	105	54	94	99	9
		February	109	102	56	78	90	11
		March	106	108	56	84	99	11

TABLE 3—*Continued*

SUBJECT	YEAR	MONTH	SYSTOLIC BLOOD PRESSURE		PULSE RATE			SCHNEIDER'S CARDIO-VASCULAR RATING
			Basal	Standing	Basal	Standing	After exercise	
C. D.	1925	April	104	103	54	89	101	10
		May	102	103	54	92	103	10
		June	104	103	53	87	93	11
		July	101	104	51	81	95	14
		August	110	115	51	82	92	14
		September	110	113	52	86	100	12
		October	108	112	53	86	103	13
		November	108	110	55	92	108	11
		December	111	109	56	95	103	10
	1927	January	107	112	54	90	96	13
E F.	1925	February	103	120	66	70	85	17
		March	98	105	66	77	96	13
		April	98	97	74	86	101	8
		May	101	99	72	87	103	10
		June	98	103	67	96	103	11
		July	100	102	63	82	101	12
		August	105	106	66	85	106	10
		September	103	106	62	77	96	12
		October	103	104	68	77	93	13
		November	101	100	62	79	96	12
		December	103	102	67	82	102	12
	1926	January	101	104	65	72	92	15
		February	103		65			
G. H.	1925	February	114	118	77	88	126	9
		March	111	109	75	95	114	7
		April	109	104	67	90	113	9
		May	109	105	70	93	116	8
		June	110	110	64	91	117	8
		July	113	108	66	93	115	7
		August	114	109	65	89	116	8
		September	113	107	69	96	115	7
		October	120	109	73	93	115	7
		November	122	109	68	98	124	6
		December	118	115	74	96	108	8
	1926	January	116	110	68	86	118	10
		February	122	117	73	88	116	8

TABLE 3—*Concluded*

SUBJECT	YEAR	MONTH	SYSTOLIC BLOOD PRESSURE		PULSE RATE			SCHNEIDER'S CARDIO-VASCULAR RATING
			Basal	Standing	Basal	Standing	After exercise	
K. L.	1926	February	109	105	71	106	122	4
		March	106	104	63	94	118	8
		April	104	102	61	98	122	7
		May	98	96	58	90	109	9
		June	97	99	58	94	109	9
		July	98	95	60	96	121	7
		August	95	92	56	94	114	8
		September	94	94	57	94	115	9
		October	95	93	58	95	118	7
		November	96	96	59	94	118	8
		December	99	95	62	103	123	6
	1927	January	99	92	64	104	128	3

From these data it seems possible to draw unhesitating conclusions in regard to the basal pulse rate and the basal and standing blood pressures; for not only are the average curves for *both* years substantially alike; but the individual averages are sufficiently concurrent to give them validity. The conclusions in regard to these functions are:

The basal pulse rate is lowest during the summer.

The basal and standing blood pressures are lowest during the spring.

Whether or not there is a seasonal variation of the pulse, standing and after exercise, and in the cardio-vascular rating, cannot be decided from these data; for there is no agreement in the results of the two years. It is reasonably certain, however, that the average curves for 1925 are meaningless because of the lack of concurrence among the four subjects during this year; why this should have been so cannot be known. On the other hand, during 1926 there was a uniformity of variation among the three subjects then being investigated, which makes the average curves for this year truly accurate reflections of the individual variations. Our opinion, therefore, would be that if there is a seasonal variation in these functions it is probably of a kind represented by the average curves of 1926; but such a conclusion must remain tentative until further evidence can be obtained.

The same considerations which were advanced in regard to the meaning of seasonal periodicity in our previous report on basal metabolism apply here. The only previous suggestion of a possible seasonal variation in the pulse rate which we have seen is that of Gustafson and Benedict (9) who found, in connection with a low metabolic rate, minimal pulse rates

during the winter. Such a state of affairs must preclude all thought of a direct climatic effect and must direct the search for the cause of these variations into a closer scrutiny of the daily habits and activities of the subjects being investigated.

SUMMARY

This report, which is a continuation of that already published (1) concerning intra-individual variations of weight, temperature and basal metabolism, deals with the basal and standing pulse rates and systolic blood pressures; the pulse rate after a standard, light exercise; and the cardio-vascular rating of physical efficiency proposed by Schneider (2).

Statistical measures are given of the intra-individual variability shown by these cardio-vascular functions; the degree of correlation between the intra-individual variations of the different cardio-vascular functions, themselves; and the correlation between intra-individual variations of basal pulse rate and systolic blood pressure with the basal oxygen consumption and the basal pulse rate with the oral temperature.

Going to sleep during a ten-minute metabolism determination is shown to reduce the pulse rate an average of two beats per minute.

Whether or not menstruation has an effect on these functions is not conclusively proven by these data; nevertheless they indicate that *1*, the standing systolic pressure is lowest during the latter part of the intermenstrual period; *2*, the pulse rates, basal, standing and after exercise, are highest in the latter part of the intermenstrual period; *3*, there is little or no uniform, average effect shown by the basal blood pressure or the cardio-vascular rating.

The basal pulse rate is lowest during the summer; the systolic pressures, basal and standing, are lowest during the spring. During 1925 there was no uniform, concurrent variation shown by the four subjects then being studied in regard to the pulse rates, standing and after exercise or the cardio-vascular rating; during 1926, on the other hand, the three subjects under investigation gave fairly uniform, concurrent evidence that these pulse rates are lowest during the summer and the cardio-vascular rating is then highest.

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THE REGULATION OF RESPIRATION

XXV. Variations in the Lactic Acid Metabolism in the Intact Brain[1]

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Received for publication December 7, 1928

It was shown that decapitation of a dog causes extremely rapid accumulation of lactic acid in the brain tissue—20 mgm. per cent per minute for the first few minutes (McGinty and Gesell, 1925). This produces not only an increase in carbonic acid but a decrease in the bicarbonate buffer base. Both changes entail increasing hydrogen ion concentration. Along such lines an application of these observations to the acid metabolism of the respiratory center and the chemical regulation of respiration was discussed (Gesell, 1925) (McGinty and Gesell, 1925). It has now seemed desirable to study lactic acid metabolism under more normal conditions and to learn whether lactic acid is formed within the intact brain under less extreme conditions of impaired oxidations and whether or not the process is reversible, that is, whether an accumulated lactic acid content of the brain would diminish on reëstablishment of oxidations as it does in muscle. The analogy drawn between the acid metabolism of muscle and of brain would thus be more completely established.

As an index to the acid metabolism of the brain, lactic acid determinations were made on the arterial blood supplying the brain and on the venous blood returning from this tissue. This was done before, during and after impairment of oxidations produced principally by occlusion of cerebral vessels and by injection of sodium cyanide. The lactic acid levels of the arterial and venous blood should indicate the direction of movement and the rate of formation or absorption of acid by the brain.

Method. Arterial and venous blood samples were taken as nearly at the same time as possible. Four determinations were made on each Folin-Wu filtrate according to the modified Clausen method of Friedemann, Cotonio and Shaffer (1927). A difference of 3 mgm. per cent or more lactic acid content between arterial and venous blood was considered of definite significance although less than three milligrams per cent difference

[1] Reported before the American Physiological Society, April, 1928 (This Journal, Proc., 1928, lxxxv, 395).

was regarded as having importance if justified by the general shape of a curve.

In a few experiments volume flow of blood was recorded using the device recently introduced by Doctor Gesell.[2] Artificial respiration was employed in every experiment but one (6-b).

Twenty-nine experiments were grouped according to the general procedure of the observations into six sections. In the first group venous samples were taken from the superior sagittal sinus[3] and in one experiment also from the femoral vein. Sodium cyanide was injected into the femoral vein.

In the second group venous samples were withdrawn from the central sinus and from the femoral vein. In four experiments cyanide was injected into the vertebral artery,[4] in a fifth experiment in the femoral artery and in a sixth experiment the results of hemorrhage were recorded.

In the third group venous blood was taken either from the sinus or from the external jugular vein. The fourth group of two experiments demonstrates the effect of repeated sampling over a long period on lactic acid content of arterial blood and on sinus and femoral venous blood.

The fifth series of six experiments consisted of a heart-lung-brain preparation.[5] Arterial samples were taken from the left subclavian artery

[2] Demonstrated at the meetings of the American Physiological Society, Ann Arbor, 1928.

[3] The skull was trephined at about the junction of the parietal and occipital bones and blood was withdrawn with a 10 cc. syringe inserted through the dura into the lumen of the sinus.

[4] Cyanide was injected with a syringe provided with a small needle which was placed into the artery so as not to interfere with the flow of blood.

[5] The chest was opened during artificial respiration and all arterial vessels from the left heart were ligated except the two common carotid and two vertebral arteries. All veins to the heart including the azygos and inferior vena cava were ligated except the two external and two internal jugular veins. A cannula in the left subclavian artery supplied blood through an artificial variable resistance to a reservoir from which the blood flowed at constant pressure directly back to the right heart via a cannula in the superior vena cava. In two experiments volume flow was recorded from a Y cannula in both external jugular veins. The venous return from the volume flow device was connected to the superior vena cava through the stump of the right external jugular vein. The volume flow instrument was a closed fluid system so that no notable resistance was encountered by the venous return to the heart. Before observations were started the neck was completely crushed with a heavy steel bar leaving the two common carotids, the two external and two internal jugular veins and the vagi above the clamp so that the head was isolated from the trunk. All of the arterial output of the left heart passed either to the head by way of the two common carotid arteries or through the external shunt of variable resistance directly to the right heart by way of a constant pressure reservoir. Venous return was limited to the two external and two internal jugular veins. Arterial blood pressure was recorded from the left subclavian artery. Heparin was used in amounts sufficient to prevent clotting.

TABLE 1

EXPERIMENT	VENOUS SAMPLE	OBSERVATION	REMARKS
1-a	Sinus	Cyanide inj. in Fem. V.	
1-b	Sinus	Cyanide inj. in Fem. V.	
1-c	Sinus and Fem. V.	Cyanide inj. in Fem. V.	
2-a	Sinus and Fem. V.	Cyanide inj. in Vert. A.	Both carotid arteries ligated
2-b	Sinus and Fem. V.	Cyanide inj. in Vert. A.	One carotid artery ligated
2-c	Fem. V.	Cyanide inj. in Fem. A.	One carotid artery ligated
2-d	Fem. V.	Hemorrhage of animal	One carotid artery ligated
3-a	Sinus	Occlusion and deocclusion of carotid and vertebral arteries	One carotid artery ligated
3-b	Rt. ext. jug. V.	Same	All minor branches of common carotids ligated. Maxillary branches of ext. jug. vein ligated
		Cyanide inj. in Car. A.	All branches of common carotids except internal and occipital arteries tied
4-a	Sinus and Fem. V.	Effect of sampling	One carotid A. tied
4-b	Sinus and Fem. V.	Effect of sampling	One carotid A. tied
5-a	Ext. jug. V.	Reduction in volume flow of arterial blood to head	H-L-B preparation. Vol. flow from both ext. jug. veins
5-b	Ext. jug. V.	Occlusion and deocclusion of carotid arteries	H-L-B preparation. Vol. flow from both ext. jug. veins. All branches of carotids except internal and occipital arteries ligated
5-c	Central sinus	Reduction of pulmonary ventilation	H-L-B preparation. Common carotid arteries intact. One ext. jug. vein ligated

TABLE 1—*Concluded*

EXPERIMENT	VENOUS SAMPLE	OBSERVATION	REMARKS
5-d	Lt. int. jug. vein	Reduction of pulmonary ventilation	H-L-B preparation. Common carotid arteries intact. One ext. jug. vein ligated. One int. jug. vein ligated
5-e	Rt. int. jug. V.	Occlusion of carotid arteries	H-L-B preparation. Lt. common carotid ligated. One ext. and one int. jug. vein ligated
6-a	Vertebral vein	Cyanide inj. in Vert. A.	Vol. flow from both Vert. V.'s. One Vert A ligated. One ext. jug. vein ligated
6-b	Vertebral vein	Cyanide inj. in Vert. A.	Natural respiration. One Vert A. ligated
6-c	Vertebral vein	Cyanide inj. in Vert. A.	Both vertebral arteries ligated
6-d	Vertebral vein	Cyanide inj. in Vert. A.	Both vertebral arteries ligated
6-e	Vertebral vein	Occlusion of vertebral and carotid arteries	Int. jug. veins ligated. One ext. jug. vein ligated

and venous samples from either the superior sagittal sinus, the internal jugular or the external jugular veins. Volume flow records were taken in two of this group of experiments.

In the sixth group of eight experiments on the intact animal venous samples were taken from the vertebral vein. Cyanide was injected through a cannula placed in one vertebral artery. Volume flow records of the joined vertebral venous flow were taken in three experiments.

RESULTS. Twenty-one experiments of the twenty-nine performed are graphically represented by curves (figs. 1 and 2) with a corresponding outline in table 1 of the general arrangements.

In each of the first series of five experiments in which cyanide was injected into the femoral vein the initial sinus lactic acid values were always below arterial by amounts varying from 0.6 to 4.8 mgm. per cent. Following injection both arterial and venous lactic acid content rapidly increased and in all of the five experiments sinus lactic acid equalled or definitely exceeded arterial content in from three to fifteen minutes. In 1-a before injection, the sinus lactic acid content was below arterial by 4.6 mgm. per cent. After 7 cc. of 0.02 N. sodium cyanide the sinus content exceeded arterial by as much as 10 mgm. per cent in 10 to 15 minutes. A partial recovery is evident. After the second injection which proved to be lethal a final sample taken about five minutes after administration

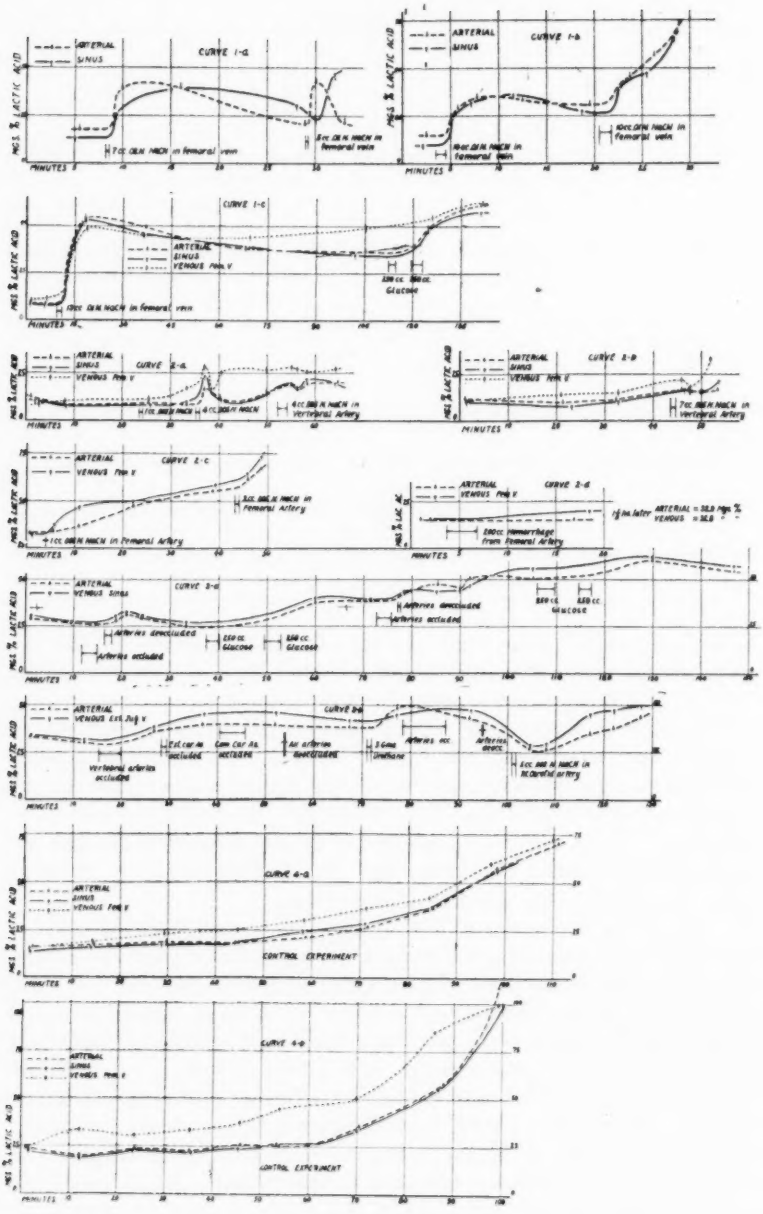
CURVE 1-a
ARTERIAL
SINUS
MGS. % LACTIC ACID
MINUTES
7 cc. D&N. HACH in femoral vein
8 cc. D&N. HACH in femoral vein
CURVE 1-b
10 cc. D&N. HACH in femoral vein
CURVE 1-c
VENOUS from V
100 cc. D&N. HACH in femoral vein
CURVE 2-a
4 cc. D&N. HACH in Vertebral Artery
CURVE 2-b
7 cc. D&N. HACH in Vertebral Artery
CURVE 2-c
8 cc. D&N. HACH in Femoral Artery
7 cc. D&N. HACH in Femoral Artery
CURVE 2-d
200 cc. Hemorrhage from Femoral Artery
CURVE 2-e
Arteries occluded
Arteries occluded
Arteries occluded
Arteries occluded
CURVE 3-a
Vertebral arteries occluded
Arteries occ.
8 cc. D&N. HACH in Femoral artery
CURVE 4-a
CONTROL EXPERIMENT
CURVE 4-b
CONTROL EXPERIMENT

Fig. 1

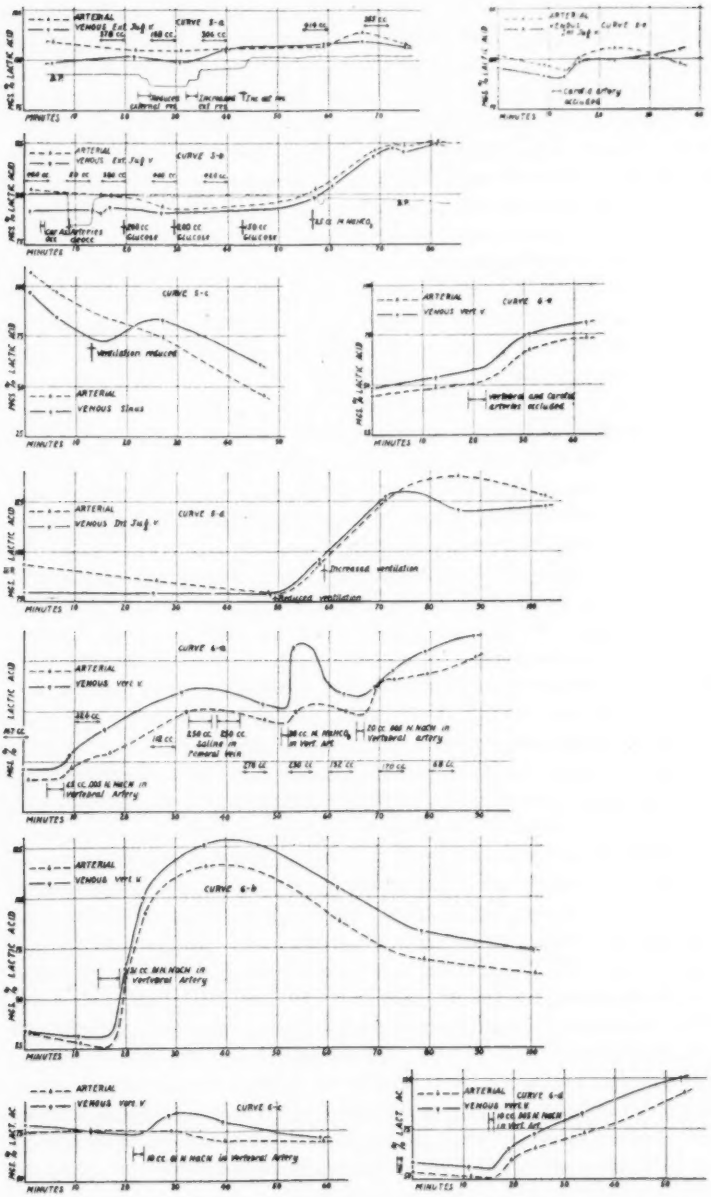
ARTERIAL
VENOUS Ext. Jug. V
CURVE 5-a
MINUTES
CURVE 5-b
CURVE 5-c
VENOUS Sinus
Ventilation reduced
CURVE 6-a
VENOUS Vert. V
Vertebral and Carotid Arteries occluded
CURVE 6-d
Increased ventilation
Reduced ventilation
CURVE 6-e
Saline in Femoral Vein
CURVE 6-b
CURVE 6-c
CURVE 6-d

Fig. 2

showed sinus content 15.6 mgm. per cent above arterial. In experiment 1-b the results are less striking but in view of the fact that previous to injection, sinus lactic acid was 4.8 mgm. per cent below arterial and in one sample seven minutes after cyanide, 3.0 mgm. per cent above arterial, the effects are similar to 1-a. A second injection caused a rapid accumulation of lactic acid with no striking variation in the relative content of arterial and venous blood. In 1-c arterial and sinus lactic acid remained at about the same comparative level. Femoral venous lactic acid remained close to the arterial and sinus values until about forty minutes after administration when it increased above the other two until after injection of glucose. A slight increase in blood lactic acid after glucose administration may also be observed in experiments 3-a and 5-b. The increase in blood lactic acid after glucose although not investigated agrees with the in-vitro experiments of Häusler (1927).

In the second group of four experiments in which cyanide was injected into a vertebral artery, the results of two are shown in curves 2-a and 2-b. As with the first group, the initial samples showed sinus lactic acid on an average to be 2.7 mgm. per cent below arterial. In 2-a, two blood samples and in 2-b, three samples all taken before injection of cyanide show that sinus lactic acid although only slightly below arterial content is consistent in its relationship to arterial values. In 2-a, injection of 1 cc. and 11 minutes later a second injection of 4 cc. resulted in an immediate increase of sinus content to 7.2 mgm. per cent above arterial followed by recovery to approximately pre-injection conditions. A further injection had only a temporary and inconclusive effect. In 2-b, injection of 7 cc. of cyanide proved lethal to the dog but from the analyses of the final samples and from the shape of the curve it seemed likely that sinus lactic acid would increase definitely above the arterial level. In a third experiment (no curve) the results were very similar to 2-a. Initially 1.4 mgm. per cent below arterial, sinus lactic acid increased to 3.0 mgm. per cent above arterial in about five minutes after a very slow injection of 9 cc. of 0.02 N. cyanide into the vertebral artery. In a fourth experiment (no curve), however, sinus values remained at about the arterial level although a general increase in both arterial and venous content resulted from 1.2 cc. 0.005 N. cyanide and later 1.8 cc. in the vertebral artery.

In 2-c cyanide was injected into the right femoral artery with a syringe. Before injection femoral arterial and femoral venous lactic acid content were about the same. Seven minutes after injection venous content increased to 9.9 mgm. per cent above arterial to return in about seventeen minutes to only slightly above arterial. In 2-d severe hemorrhage of the animal caused a more rapid appearance of lactic acid in venous blood as compared to arterial blood with approximate recovery $1\frac{1}{2}$ hours later.

In the third series of experiments cranial arteries were occluded and de-

occluded as indicated in curves 3-a and 3-b. In the first experiment (3-a) occlusion of the vertebrals and of the carotid artery that was intact, in the first observation, caused only a slight increase in sinus lactic acid over that of arterial but the results of the second occlusion were more striking. Before occlusion sinus and arterial lactic acid content were about the same. Twenty-five minutes after de-occlusion sinus exceeded arterial lactic acid by 4.9 mgm. per cent to be followed by partial recovery. In 3-b arterial and external jugular values initially at the same level diverged after occlusion of the arterial supply with venous exceeding arterial lactic acid content by 6.8 mgm. per cent. A slight tendency toward recovery is evident. In the last observation of 3-b cyanide was injected into the right common carotid with the external carotid and minor branches ligated. The rapid increase of jugular lactic acid content over that of arterial is in line with results obtained by injection into the vertebral arteries.

Curves 4-a and 4-b represent semi-control experiments demonstrating the effect of continual hemorrhage necessary for sampling. The differences in arterial and sinus bloods were small and varied only slightly throughout the experiment. Femoral venous lactic acid content with the exception of the initial samples remained consistently higher throughout. The inconsistency of venous content in the final sample of 4-b cannot be explained.

In six experiments with the heart-lung-brain preparation lactic acid of venous blood, whatever its source—external jugular, internal jugular or sinus—was always consideraby lower than arterial lactic acid at the beginning of the experiment. In 5-a external jugular lactic acid was 10.4, 3.2 and in one sample taken during the period of diminished blood flow, 5.8, an average of 6.4 mgm. per cent below arterial content. Approximately five minutes after the diminution in carotid volume flow venous and arterial lactic acid were at about the same level. In a final pair of samples, as recovery starts, venous lactic acid content again falls below arterial. The thin solid line of the graph is a record of the blood pressure as recorded from the left subclavian artery. The volume flow from the external jugular veins calculated for five minute periods appears on the graph. In 5-b the arrangements were the same except that all branches of the common carotids except the internal and occipital arteries were ligated. The carotid arteries were occluded and de-occluded as indicated. Volume flow from the external jugular veins also appears on the record. Previous to occlusion venous lactic acid was below arterial by 10.7 mgm. per cent and in two samples immediately following the fall in blood pressure 8.7 and 5.0 mgm. per cent respectively. Twenty minutes after de-occlusion jugular venous lactic acid remained below arterial but the difference between the two had fallen to 3.1 mgm. per cent. Glucose and $NaHCO_3$

caused a considerable increase in blood lactic acid agreeing with the observations of Macleod and Knapp (1918–19). In the third experiment, (5-c), attempted volume flow measurements from the central sinus failed because of difficulties in blood clotting. In this experiment ventilation was radically reduced at the point indicated on the curve. In two samples preceding the decrease in lung ventilation, sinus lactic acid was decidedly below arterial with both bloods showing a rapid diminution in lactic acid content. The preparation was thought to be greatly overventilated during this period. After the decrease in ventilation arterial lactic acid continued to fall at about the same rate but sinus lactic acid content increased or at least failed to decrease for a time. However, with readjustments and without further altering ventilation the lactic acid content of both bloods continued to decrease with sinus values at this time above arterial. In a fourth experiment (no curve) the arrangements were as in the third with the same difficulties in sinus flow as previously encountered. Reduction of ventilation actually caused heart failure so that manual message of the heart was necessary to maintain blood flow until the cardiac center sufficiently recovered. Before reduction of pulmonary ventilation, in two samples, sinus lactic acid content was 6.4 and 2.8 mgm. per cent below arterial. Following reduction sinus and arterial content were about identical. With the final sample twenty minutes later, sinus lactic acid was 2.8 mgm. per cent above arterial. In 5-d volume flow was recorded from the left internal jugular vein, the right being ligated. The initial pair of samples showed venous to be 13.8 mgm. per cent below arterial lactic acid, a second pair 5.6 mgm. per cent below and in a third pair at the same level. Reduction in ventilation from 250 to 100 cc. per stroke of the pump for ten minutes caused a rapid increase in lactic acid content of both bloods. In a sixth sample an apparent recovery begins with venous again falling much below the arterial level. In this experiment the volume flow record was somewhat unsatisfactory because of partial clotting in the volume flow recorder but it was evident that reduction of ventilation actually caused an increase in the flow of blood from the internal jugular vein.

In the next series of eight experiments circulation was intact. Volume flow records of vertebral venous flow were taken in the first three experiments. In all of this series venous lactic acid was initially higher than arterial. A study of the absolute values shows, however, that in every experiment but one, both arterial and venous lactic acid contents were above 40 mgm. per cent as contrasted with the first fourteen experiments using the intact animal, in which arterial content varied between 8.8 to 40.0, an average of 21.2 mgm. per cent and venous values varied between 8.6 to 37.4, an average of 19.7 mgm. per cent. In this final group arterial content varied from 33.4 to 74.4, an average of 53.2 and venous lactic acid varied from 34.0 to 79.1, an average of 57.2 mgm. per cent. These

figures seem to indicate that conditions of impaired oxidations in the body existed before the observations were started. That brain tissue as well suffered from disturbed oxidations is especially likely in view of the fact that not only was considerable time necessary to prepare the experiment but in placing a cannula in the vertebral artery which in itself diminished the blood flow to the brain, considerable interference with carotid flow was unavoidable. Notwithstanding the difficulties encountered in preparation it was desirable to introduce the cyanide through the vertebral artery and thus insure a more certain localization of cyanide effect on brain tissue.

In six of the eight experiments of this group injection of cyanide was invariably followed by a more rapid and greater increase in venous content of lactic acid than in arterial content. Since the results were so uniform in character only four curves are given (6-a, 6-b, 6-c and 6-d). Previous to injection of 25 cc. of 0.005 N. cyanide (see curve 6-a) the volume flow was 167 cc. in five minutes. During and immediately following injection, volume flow increased to 324 cc. in a five-minute period; twenty minutes after injection volume flow had fallen again to 112 cc. Venous lactic acid content increased from 5.4 mgm. per cent above arterial before injection to 11.3 mgm. per cent above arterial ten minutes after injection. A second injection of cyanide yielded similar results both in lactic acid changes and in volume flow of blood. In experiment 6-b with natural respiration intra-arterial injection of 31 cc. of 0.01 N. cyanide resulted in a much greater relative increase in venous lactic acid than in arterial content. Beginning recovery in this experiment is quite evident. In 6-c and 6-d results are similar with evidence of complete recovery in 6-c. In one of this series of experiments (6-e), the vertebral and carotid arteries were occluded. Before occlusion in two samples vertebral venous lactic acid content was 4.1 and 5.6 mgm. per cent respectively above arterial content. Following occlusion which was maintained venous lactic acid increased to 8.2 and 7.9 mgm. per cent above arterial in two samples. In another experiment, (no curve), suspended respiration for 40 seconds failed to alter the relationship between arterial and venous values although both increased in absolute content.

Discussion. Of twenty-nine experiments, the initial blood samples indicated that lactic acid content of venous blood returning from the head was definitely below arterial content in twelve experiments, approximately the same in ten and above arterial content in seven experiments That the greater number of samples of venous blood contained initially less lactic acid than arterial blood was true whether the samples were taken from the superior sagittal sinus, the external jugular veins or the internal jugular veins. In those experiments in which venous samples were taken from the vertebral veins the initial venous lactic acid content was definitely

higher than arterial in six cases but the absolute values when compared to those of the first group indicated that a certain degree of impaired oxidations existed at the beginning of the observations so that lactic acid formation in brain substance was in excess of lactic acid absorption. Whether the fact that vertebral venous blood contained initially more lactic acid than arterial blood is of any further significance or, as suggested, merely coincidental with a preëxisting decrease in oxidations, cannot be said. The latter view is more tenable. Although vertebral blood can be considered to be largely from brain tissue, sinus blood as withdrawn, represents almost wholly drainage from cerebral vessels and furthermore sinus values initially were more or less consistently below arterial values.

Himwich, Koskoff and Nahum (1928) report in a preliminary paper that certain organs at rest add lactic acid to the blood and that other organs, particularly the liver, remove it from the blood. From the foregoing experiments it seems, considering the evidence as a whole, that with sufficient oxygen supply to the lungs, adequate cerebral circulation and normal utilization of oxygen by the brain cells, lactic acid is absorbed there and utilized as a food substance. Conversely, with impaired oxidations as with curtailment of oxygen supply, decrease in arterial flow to the brain or by disturbance of the oxidative mechanism during cyanide poisoning, lactic acid production becomes in excess of utilization. This is evidenced by its greater content in venous than in arterial blood circulating brain tissue. This conclusion is in line with work of Barr and Himwich (1923) in which they demonstrated that an active limb adds lactic acid to its blood whereas a limb at rest removes lactic acid from the blood.

Another factor may be considered. The quantity of blood flow through a tissue determines not only the rate at which nutritive material is carried to the cells but the rate at which waste or excessive metabolites are removed from the cells. If the comparative lactic acid levels of arterial and venous blood indicate the direction and gradient of movement of lactic acid, whether from the blood into the cells or from the cells into the blood, then if one multiplies the differences in lactic acid content between arterial and venous blood by the volume flow of blood the product represents the rate of utilization or the rate of formation of lactic acid by the tissue. Although the experimental difficulties were too great to obtain quantitative figures in many experiments, the results of one or two are worthy of a more detailed analysis. In cyanide experiments there is ample evidence that volume flow of blood is increased during and immediately following injection into cerebral vessels (Bernthal, Bronk, Cordero and Gesell, 1928). Since at the same time venous lactic acid content increases out of proportion to the increase in arterial content, lactic acid production is taking place in a relatively greater amount than that removed. In the

first observation (6-a) previous to the injection of cyanide, when the venous lactic acid content was 5.4 mgm. per cent above the arterial content and the volume flow of vertebral venous blood was 167 cc. in five minutes the proportionate formation of lactic acid by the brain amounted to 0.054 times 167 or 9.0 mgm. for five minutes. Five minutes after injection when the volume flow was 324 cc. and venous lactic acid exceeded arterial by approximately 10.0 mgm. per cent, relative lactic acid formation was approximately 32.4 mgm., a proportional increase of more than 250 per cent over that before injection. Later with the volume flow at 112 cc. and venous lactic acid exceeding arterial by 12.5 mgm. per cent, lactic acid formation had fallen to 14.0 mgm. in five minutes, only 55 per cent above that before injection. In this experiment assuming that the brain weighed 75 grams and that the volume flow of blood from the two vertebral veins represented 50 per cent of the drainage from brain substance, it may be calculated that lactic acid formation increased from an initial value of 5.3 mgm. per 100 grams of brain tissue per minute to 17.3 mgm. on injection of cyanide, to decrease 15 minutes later to 7.5 mgm. per 100 grams of tissue per minute. These values compare favorably with those of the decapitation experiments in which lactic acid accumulated for the first few minutes at a rate of 20 mgm. per minute per 100 grams of brain tissue.

In 5-a, a heart-lung-brain preparation, the volume flow from the external jugular veins represents only a fraction of that perfusing brain tissue but since the brain makes up a large part of the tissue of the head the results of this experiment may indicate at least relatively the conditions existing in brain tissue. Previous to the fall in blood pressure the volume flow was 378 cc. in 5 minutes with venous lactic acid content below arterial by 5.0 mgm. per cent, showing an absorption of 18.9 mgm. of lactic from arterial blood during a 5-minute period. During the experimental period of diminished flow (108 cc. in five minutes) lactic acid absorption had fallen to approximately 5.4 mgm., only 28.5 per cent of that absorbed in the same time previous to the decreased arterial flow. With reëstablishment of arterial flow when the venous flow was 306 cc. in 5 minutes, absorption continued but had decreased still further to 4.6 mgm., 24.5 per cent of that before the diminished arterial flow. Twenty minutes later with a volume flow of 414 cc., lactic acid formation slightly exceeded its rate of absorption so that at this time about 4.1 mgm. lactic acid above the arterial content appeared in the venous blood during the 5-minute period. Recovery of the oxidative mechanism follows and lactic acid formation gives way to absorption approximately 40 minutes after the experimental observation. Absorption at this time amounted to about 14.0 mgm. in 5 minutes, 74 per cent of the initial rate.

In experiment 5-b with only the internal carotid and occipital arteries supplying blood to the head, external jugular venous blood may be said

to contain a greater proportion of blood from brain tissue. In this experiment an initial rate of absorption of 45.0 mgm. in 5 minutes decreased with the fall in arterial flow to 4.0 mgm. lactic acid. Four minutes later the rate of absorption returned to about 20.9 mgm. and remained approximately at that level. Lactic acid absorption in brain tissue decreased comparatively by about 50 per cent as a result of a temporary decrease in volume flow of arterial blood.

Recovery from disturbed oxidations and return to normal conditions was evident in only a few experiments. However, judging from the trend of the curves in a larger number of observations it is believed that unless injury to the oxidative mechanism was too severe, recovery is possible.

SUMMARY

As an index to the lactic acid metabolism in the brain of an intact animal, lactic acid determinations were made on arterial blood supplying the brain and on venous blood returning from the brain. To obtain a more accurate index of the lactic acid metabolism volume flow of venous blood was recorded in a few experiments.

In twelve of a total of twenty-nine experiments, venous blood from either the superior sagittal sinus, the external jugular vein or the internal jugular vein contained initially less lactic acid than simultaneous samples of arterial blood indicating a movement of lactic acid from the arterial blood into the brain tissue.

In ten experiments venous and arterial lactic acid content were at the same level showing that a condition of equilibrium between lactic acid absorption and lactic acid formation prevailed.

In seven experiments venous blood contained initially more lactic acid than arterial blood indicating an outward movement of lactic acid from the brain cells into the blood stream.

Impairment of oxidations in the brain by injection of sodium cyanide, partial or complete occlusion of the cerebral blood supply and diminished pulmonary ventilation resulted in an increased content of lactic acid in venous blood over that of arterial blood.

It was demonstrated that injection of sodium cyanide into the vertebral artery may cause a relatively increased production of lactic acid in brain of over 250 per cent. In another experiment a fall in blood pressure resulted in decreased absorption of lactic acid from the arterial blood to 24.5 per cent of the initial rate, with partial recovery to 74 per cent of the initial rate. A temporary reduction of arterial blood supply in another experiment caused a 50 per cent reduction in lactic acid absorption from arterial blood.

It is believed that with normal oxidations in brain tissue, lactic acid is absorbed from the arterial blood and utilized by the brain cells.

With impaired oxidations there occurs an excessive production of lactic acid in the brain cells with an outward diffusion into the blood stream.

The analogy between lactic acid metabolism of muscle and lactic acid metabolism of brain tissue with its relationship to the chemical control of respiration is believed to be more firmly established.

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PHYSIOLOGY OF THE CORPUS LUTEUM

II. Production of a Special Uterine Reaction (Progestational Proliferation) by Extracts of the Corpus Luteum

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Received for publication December 19, 1928

Among the numerous functions attributed to the corpus luteum, the best attested is that based upon the work of Fraenkel, Loeb, Ancel and Bouin, and others.[1] The results of this work indicate that following ovulation and formation of the corpus luteum the endometrium undergoes histological changes leading to the production of a special state, which may be called progestational proliferation. In this state the uterus becomes enlarged and hyperemic; its epithelium, both superficial and glandular, undergoes mitotic proliferation, and the crypts and glands increase their complexity of ramification until in cross-section a very characteristic picture is produced. This condition occurs only in the presence of recent corpora lutea in the ovaries and can be prevented if both ovaries or all the corpora lutea are removed soon after ovulation (Bouin and Ancel). At the same period of the reproductive cycle the endometrium of certain mammals is known to be in a special condition by which placentation is facilitated, since it responds even to traumatic injury by producing decidual tissue at the site of stimulation; this reaction is prevented by removal of the corpora lutea soon after ovulation (Loeb). Finally, if both ovaries or all the corpora lutea of pregnant rabbits are removed at any time during the first week of pregnancy, implantation does not occur, and the embryos are lost (Fraenkel).

In the first paper of the present series (Corner, 1928) the methods of Ancel and Bouin and of Fraenkel were made the basis of a series of experiments planned to test anew the mechanism by which the endometrium is induced to undergo progestational proliferation, and to determine the exact fate of the embryos when the proliferation is prevented. In this paper (which should be read as an introduction to the following discussion) it was shown, in confirmation of Ancel and Bouin, that removal of both ovaries or of the corpora lutea only, by surgical intervention 14 to 20 hours

[1] For fuller discussion of the work quoted in this paragraph, see Corner (1923, 1928).

after mating (when the fertilized ova have been in the Fallopian tubes 4 to 10 hours) causes total failure of progestational proliferation, the endometrium remaining in the same histological state as at the time of mating. Under these conditions the embryos may develop for about three days in the tube, reaching the uterus on the fourth day, as in normal pregnancy, but they do not survive beyond the fourth day. This fact indicates that progestational proliferation is necessary not only for implantation, but also for the nutrition or protection of the free blastocysts during the period of four days between arrival in the uterus and implantation.

The results recounted in the foregoing paper, which indicate clearly that the corpus luteum is an organ of internal secretion acting upon the endometrium on behalf of the embryos, led us to attempt the preparation of extracts of the corpus luteum capable of producing progestational proliferation of the endometrium. At the same time these experiments have placed in our hands a standardized method for testing the extracts. For the test (which will be described in detail below), a doe is mated and 18 hours later is subjected to removal of both ovaries and to the excision of a small portion of the uterus. The extract is administered for five days, and on the 6th day after mating the animal is killed, the embryos recovered if present, and the uterus submitted to histological examination and comparison with the specimen removed at the time of castration.

Using this test we have been able to prepare alcoholic extracts of the corpora lutea of swine which produce in spayed rabbits a condition of the uterus identical with normal progestational proliferation. Under the influence of these extracts the uterus undergoes general growth (most marked in the younger does and those which have not recently borne young), its vessels become moderately engorged with blood, and the endometrial glands and crypts undergo the characteristic proliferation (fig. 2). To the eye and under the microscope the condition of such a uterus can in no way be distinguished from that of normal pregnancy of the fifth or sixth day, except that after large doses the proliferation may be even greater than in normal pregnancy (fig. 3). That the condition of the uterus produced by these potent extracts of the corpus luteum is functionally as well as morphologically identical with normal early pregnancy will be shown in the following paper, the third of the series, in which the survival of the embryos of the treated rabbits is described (Allen and Corner, 1929).

Details of the test. An adult doe, preferably one which has been isolated for a month or more, is mated to one or two bucks and insemination proven by discovery of spermatozoa in vaginal smears made immediately after mating.[2] The animal is thereupon again isolated until conclusion of the

[2] For details of the physiology of reproduction in the rabbit, and for practical information as to mating, see Hammond and Marshall (1925).

experiment. About 18 hours after mating the animal is anesthetized with ether and the abdomen is opened under aseptic precautions. If ruptured follicles are found in the ovaries as expected, the ovaries are removed. It is our practice to clamp the ovarian pedicle tightly with a small artery clamp, to transfix and ligate the pedicle with black silk on the proximal side of the clamp, and to cut away the ovary along the distal side of the clamp. Bleeding points are carefully controlled by ligature with silk, avoiding ligation or kinking of the Fallopian tube. Complete removal of the ovaries (to insure total ablation of the corpora lutea) is of course essential to accuracy of the test. A piece of the uterus about one or two centimeters long at the middle of the left cornu is removed between ligatures after the mesometrial vessels supplying the excised portion are tied, and finally the cut ends of the uterus are approximated by tying together the ends of the two uterine ligatures. The portion of the uterus thus removed is preserved in Bouin's fluid for use as a histological control. The incision is closed in two layers with silk. The extract is administered subcutaneously in the dorsal region immediately following the operation and once daily thereafter until five doses have been given. The animal is killed 5 days after the operation, one day after the final dose, and the genital tract and other tissues are removed for examination. The sites of the ovaries are carefully searched for surviving ovarian tissue and any suspicious tissues are examined under the dissecting microscope and if necessary preserved for sectioning. The right uterine cornu, the left cornu above the ligature, and the two Fallopian tubes are successively cut from the reproductive tract and washed out into watch-glasses with normal salt solution by means of a hypodermic needle and syringe, in order to obtain the embryos. Finally the parts of the genital tract are fixed in

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Fig. 1. Control section of uterus of rabbit X49, 18 hours after mating. ×7. Compare with figure 2.

Fig. 2. Uterus of rabbit X49 after 5 days' treatment with corpus luteum extract. Typical proliferation. ×7.

Fig. 3. Uterus of rabbit X52 after 5 days' treatment with corpus luteum extract. Extreme proliferation. ×7.

Fig. 4. Uterus of rabbit X63, showing partial proliferation as a result of 5 days' treatment with one-half the effective minimal dose of corpus luteum extract. ×7.

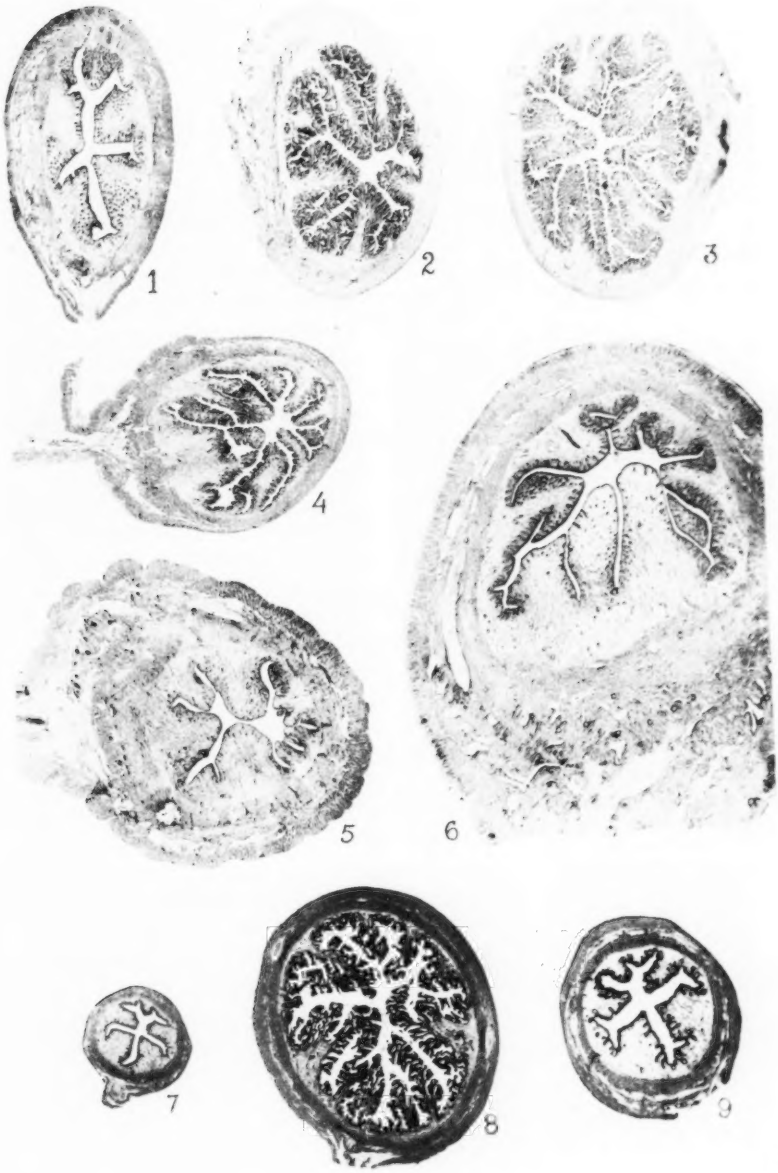
Fig. 5. Control section of uterus of rabbit X59, 18 hours after mating. ×7. Compare with figure 6.

Fig. 6. Uterus of rabbit X59 after 5 days' treatment with extract of follicular fluid (50 rat units). Growth but no proliferation. ×7.

Fig. 7. Control section of uterus of rabbit X21, immature. ×7. Compare with figure 8 and figure 9.

Fig. 8. Uterus of rabbit X21 after 5 days' treatment with corpus luteum extract, showing the typical proliferation sometimes obtained in the immature rabbit. ×7.

Fig. 9. Uterus of rabbit X22 (a litter mate of X21) after 5 days' treatment with extract of follicular fluid. Growth but no proliferation. ×7.



Bouin's fluid for sectioning. A fairly accurate provisional estimate of the result can be obtained without waiting for paraffin sections, by examining with a dissecting microscope in a strong light the surfaces of razor-cuts made through the specimen after a few hours' hardening in the fixing-fluid. The result of the test is measured by the degree of progestational proliferation induced in the uterus, according to a standard described below (p. 332).

The foregoing routine has been used in all experiments in which it was desired to study the fate of the embryos as well as the reaction of the uterus; but we have found that the extracts act equally well in animals in which mating has not been followed by ovulation, as occasionally happens. Presumably mature does will react to the extract whether mated or not, but our experience on this point is limited to one experiment upon an animal which was not mated, and in which the ovaries contained only large follicles. In this case the uterus responded fully to the extract. Hisaw (1927) states that the effects of his corpus luteum extracts can only be elicited in the guinea pig after previous preparation with oestrin. It will be remembered that the rabbit is peculiar in that large follicles ready for maturation are more or less continuously present in the ovaries. We recommend at present, however, that mated does be used in repeating these experiments and testing new extracts, in order to secure animals in exactly comparable condition as regards the reproductive system.

Preparation of the extracts. The material for the extracts is obtained in a fresh condition, all the ovaries used being received within 7 hours after the death of the animal. We are indebted to the Rochester Packing Company for their coöperation in the furnishing of this material.

Upon receipt of the ovaries at the laboratory they are separated into three groups, one group comprising those ovaries which contain large follicles and only retrogressing corpora lutea, another group which comprises ovaries bearing recently ruptured follicles and corpora lutea of all stages of the cycle and of pregnancy except those in retrogression (which are included in the first group)[3] and a third group which comprises ovaries with no follicles and no corpora lutea, together with those containing large ovarian cysts. This last group is discarded since it contains only nonfunctional and pathological ovaries. The follicles from the first group are tapped by aspiration, always on the day on which they are received, and the follicle fluid thus obtained is preserved by the addition of two volumes of 95 per cent alcohol. From this material our extracts of follicle fluid are made. The corpora lutea from the second group are dissected from the ovary, likewise on the same day that they are received and usually while

[3] Those who are unfamiliar with the normal appearances and dimensions of the follicles and corpora lutea of the sow are referred to Corner (1921).

they are still warm, ground up with a meat grinder and preserved by the addition of two volumes of 95 per cent alcohol. The material is stored in this condition until it is desired for use, remaining active for at least ten days.

The method used in the preparation of the extracts is a modification of that used by Herrmann (1915). It consists essentially of the extraction of the lipids with hot alcohol in an extractor of the type devised by Clarke, as modified by Dr. W. R. Bloor and described by Sperry (1926), and the subsequent precipitation and removal of the phospholipids from an ethereal solution by the addition of acetone.

The procedure in detail for the preparation of a typical corpus luteum extract is as follows: approximately 1500 grams of tissue, which have been preserved as described, are filtered through a bag or gauze and the residue is divided into equal parts and each part extracted five times with hot alcohol in a Bloor extractor, each extraction lasting for one hour. About 250 cc. of 95 per cent alcohol are used for each extraction. The mass of tissue in the bags is broken up by kneading between extractions to facilitate the penetration of the alcohol fumes. By this method the lipids are completely removed in a fairly short time and none of the extracted material is heated above the boiling-point of alcohol for more than one hour. The extraction alcohol contains a quantity of insoluble debris but this is not filtered off since it is removed by subsequent treatment. The alcohol is distilled off by heat with the aid of the diminished pressure obtained with a good water pump. The extraction and preserving alcohols are distilled in separate flasks since the preserving alcohol is apt to foam by reason of its greater content of water. If a good vacuum is obtained the temperature rarely goes above 60°C. and usually not above 45°C. When the solvent has been almost completely removed, the distillation is stopped to avoid overheating. The residues obtained from the extraction and preserving alcohols are combined and extracted five times with peroxide-free ether, 500 cc. being used for the first extraction and 200 cc. for each of the remaining extractions. The ether solutions from the above extractions are combined and the volume reduced to about 100 cc. by vacuum distillation. To this solution 4 volumes of acetone are added. A heavy flocculent precipitate consisting chiefly of phospholipids is formed and is removed by decanting or centrifuging. The precipitate is redissolved in ether and reprecipitated by acetone five times. After the first or second precipitation the precipitate does not completely dissolve in ether, a fine white precipitate remaining. It is not necessary, however, to remove this precipitate, which consists chiefly of sphingomyelin, since it is eventually removed along with the phospholipids by the discarding of the precipitate obtained by the addition of acetone. The acetone-ether solutions are combined and the solvents removed by vacuum distillation on the steam bath. A heavy oil is ob-

tained which is removed from the distilling flask to a centrifuge tube with the aid of about 50 cc. of ether. The solution is centrifuged, thus removing most of the water which is carried through the above solvents together with any ether-insoluble material. The ether layer is poured or pipetted off and the ether is then removed by a current of warm air. The resulting oily mixture is semi-fluid and may be administered without the addition of a vehicle, providing it is made fluid by immersing the container in warm water for a short time. The yield is about 30 grams, representing 20 grams per kilogram of fresh tissue.

The method described has been used in the preparation of all of our corpus luteum extracts and most of our follicular and placental extracts. With the extracts of follicular fluid, the amounts of the various solvents and the number of extractions have of course been reduced because of the smaller amount of material. "Mazola" (commercial maize-oil) has been used as a vehicle for both the follicular-fluid extracts and the placental extracts. The testing of the extracts for the presence of oestrin has been carried out on spayed rats, the animal receiving the amount to be tested in one subcutaneous injection rather than in three as suggested by E. Allen and Doisy (1924). The yield in rat units from the human placenta has been between 250 and 375 r.u. per kilogram; from follicular fluid between 100 and 200 r.u. per kilogram; and from corpus luteum between 10 and 20 r.u. per kilogram.

We are at present unable to make definite statements regarding the chemistry of the active substance of the corpus luteum. The activity is not completely destroyed by temperatures of about 100°C., since one of the extracts was heated to this temperature for a short time by accident. It is comparatively stable in alcoholic solution, at least for ten days and probably much longer. The activity of the oily product used for the injections may decrease on standing, but we have obtained a positive result by the injection of 1 cc. per day for five days beginning when the extract was 23 days old. The extract contains little or no protein and is non-toxic in the dosage given by us, namely, 0.12 cc. to 2.0 cc. daily. Young animals have occasionally lost weight, but when the dosage has not been excessive, they have maintained their weight and some have even gained.

✓The test is considered fully positive if at the time of necropsy the endometrium has attained throughout both cornua a condition similar to that of the uterus at the 8th day of normal pregnancy (as seen in sections taken from the undilated portions of the uterus between the implantation sites). A typical section which we have used as our standard is illustrated in figures 3 and 5 of the first paper of this series (Corner, 1928).

In future work, as we proceed to quantitative estimation of the potency of the extracts, we propose to use such a standard to define a "rabbit unit," which will be the minimum dose of an extract which suffices, when divided

into 5 daily doses, to alter the uterus of a doe weighing 3 to 4 kgm., under the specified experimental test, to a state equal to that described in the preceding paragraph.

In a preliminary attempt to determine the minimal effective dose, we have found one extract to be potent when given in doses of 0.12 cc. per day for five days, but to be ineffective when given in doses of 0.06 cc. In this experiment, therefore, 0.6 cc. was required for one rabbit unit, and a yield of 32 rabbit units per kilogram of fresh tissue was obtained.

Number of experiments; control observations. Tests of the extracts have been made with 5 consecutive preparations, administered to 8 animals under the specified test conditions, and to two others in which ovulation did not take place. All these experiments have given positive results; there have been no failures except in a few animals (not included among those mentioned) in which the dosage has been deliberately reduced below 0.12 cc. daily, in a preliminary effort to determine the minimum effective dose. In addition to these tests, we have in our records a sixth potent extract tested only upon the two rabbits (X56 and X57) described in the third paper of the series (Allen and Corner, 1929) and several potent extracts tested only upon immature rabbits, as explained in a subsequent paragraph.

The histological result has in several of these experiments exceeded the standard set; in other words, we have produced in 5 days with 5 to 10 cc. of the extract a greater degree of proliferation than occurs in the normal animal by means of 8 days' action of her own corpora lutea. Figure 3 illustrates such an extreme effect.

As to control observations, it may be said in the first place that the foregoing experiments are controlled by the results tabulated as groups I and III of the preceding paper (Corner, 1928), including 7 animals placed under experimental conditions identical with those of our test, and 6 others in which the corpora lutea alone were excised, making in all thirteen consecutive experiments in which progestational proliferation failed to occur in untreated does after complete ablation of the lutein tissue.

It next becomes necessary to consider whether the production of progestational proliferation is a specific property of the corpus luteum. To study this question we have made extracts of follicular fluid from the ovaries of swine and of human placental tissue. We have already mentioned that our corpus luteum extracts contain small quantities of the so-called oestrin or follicular hormone, amounting to 0.5 to 1 rat unit per cc. of the extract, so that the animals which received these preparations received in the five days' treatment from 2.5 to 5 rat units of oestrin. It seemed possible that this substance or some other unknown and perhaps widely distributed ingredient of the genital tissues might be responsible for the effects obtained in our tests.

Follicular fluid was collected as mentioned above from carefully selected

follicles of swine, choosing only normal follicles 6 to 10 mm. in diameter, in order to avoid cyst-fluid and the contents of cystic corpora lutea The material was treated exactly as was the corpus luteum substance, by extraction with hot alcohol and subsequent removal of the phospholipids. The oily residue thus obtained was diluted with "Mazola" and tested on rabbits by the usual method. Three such extracts were made and administered to 5 rabbits, each of which received a total of 40 to 100 rat-units of oestrin in the five doses. In none of these experiments did the uterus undergo progestational proliferation (figs. 5, 6).

Fresh human placentas, obtained from the obstetrical clinic of the Strong Memorial Hospital through the courtesy of Prof. Karl M. Wilson, were prepared in the same way. The extract from 2065 grams of tissue was divided between 2 rabbits. Owing to the large amount of oestrin in the human placenta, we were able to administer to one of these animals as much as 400 rat units in 5 days, and to the other 100 rat units. The effect of these extracts was the same as that of the follicular fluid. It thus appears that the human placenta at term contains less than 1 rabbit unit of the corpus-luteum potent substance per kilogram of fresh tissue,—a figure in decided contrast to the demonstrable presence of at least 360 rat-units of oestrin per kilogram in the same extract.

These results demonstrate that progestational proliferation cannot be produced by oestrin ("follicular hormone," "female sex hormone"), a conclusion already reached by Loeb and Kountz (1928), who showed that various extracts of the oestrus-inducing type administered to guinea pigs bring about definite changes of the genital organs akin to those of normal oestrus, but do not produce the uterine reaction characteristic of early pregnancy and the corpus luteum phase of the cycle. Ebhardt (1928) has reported exactly the same finding. The experiments of Asdell and Marshall (1927) though less extensive than those of Loeb and Kountz, bear even more directly upon this question, since they were performed upon rabbits, in which treatment with extracts containing oestrin proved, as in our work, to bring the uteri of the animals up to the state normal at the time of mating, but not into the progestational state.

Although we have not been able to elicit progestational proliferation with our extracts of the placenta or with the follicular fluid, we are not yet prepared to assert that the progestational substance is not present in these tissues. It is possible that there is an antagonistic relation between oestrin and the progestational substance. This has been suggested already by the work of Margaret Smith (1926) who found that oestrin administered to rats during the first five days of pregnancy terminated gestation. Such an antagonism, if it affects the endometrium directly, may well lead to the masking of small amounts of progestational substance by the large amounts of oestrin present in the placenta and follicular fluid.

It seems probable, however, from our experiments that the progestational proliferation is caused by a specific hormone, differing from oestrin, which is elaborated by the corpus luteum; and this is our present working hypothesis.

General growth effect. Many previous workers with extracts of genital tissues have observed, as a result of their preparations, a general growth of the uterus and vagina in immature rabbits. In this type of growth both muscular wall and endometrium are involved, but there is little or no increase in number or complexity of the glands, so that the endometrium reaches at most the adult resting stage (fig. 9). A similar effect has been found to occur in adult does after castration, when by the administration of extracts "castrate atrophy" is prevented and the uterus maintained at its normal size. Such general growth of the uterus has been found by Fellner (1913), Seitz, Wintz, and Fingerhut (1914), Frank and Rosenbloom (1915), Herrmann (1915), E. Allen and Doisy (1923, 1924) and others, to be produced by extracts from various sources, including the corpus luteum, follicle fluid, placenta, etc. So far as we are aware, all the extracts which produce such an effect are either known to contain oestrin, or are made by methods which at least should not destroy it. Hartmann (1926), using carefully purified preparations, concludes that one and the same substance is responsible for oestrus and for uterine growth, and the experiments of Laqueur, Borchardt, and de Jongh (1927) are in agreement with this, suggesting moreover that the uterus of the immature rabbit is a very sensitive indicator for this substance, since it reacts to a dose even smaller than that required to elicit oestrous changes in the rat's vagina.

The significance of this reaction in connection with our corpus luteum extracts will appear in the next paragraph.

Action of corpus luteum extracts upon immature rabbits. Because of the early experiments of Herrmann (1915) to which fuller reference will be made below, our first corpus luteum extracts were tested upon immature rabbits ranging from 600 grams to 1500 grams weight (about 8–12 weeks old). At this age the ovaries are infantile and the uterine cornua are only about 1 mm. in diameter. The test consisted simply in subcutaneous administration of the extracts in various doses (1 to 8 cc. daily for 5 to 10 days). No sample of the uterus was taken in advance for histological control, but the experiments were amply controlled by the use of litter-mate sisters which were given other extracts, or injections of "Mazola," or were left untreated (fig. 7). The effects of the corpus luteum extracts were remarkably variable. In all, 23 young rabbits were treated with doses which we now know would have been effective in adults. Of these, 5 underwent typical endometrial proliferation comparable in degree (allowing for smaller size of the uterus) to that attained in adults under the most favorable conditions (fig. 8). In these animals the uteri also underwent

remarkable general growth, increasing three- to five-fold in diameter and ten-fold or more in weight. In section such a uterus could scarcely be distinguished from that of an adult doe five or six days pregnant. In 8 animals, on the other hand, we obtained no growth or proliferation at all; in 10 we obtained moderate growth and slight proliferation. In this last group we were surprised to find very often a localized response of the uterus, such that parts of one or both cornua were enlarged and the endometrium proliferated, while other parts remained in the infantile condition,producing a very bizarre appearance of the uterus.

These unaccountable variations in the action of the extracts caused us much perplexity until by a process of elimination we arrived at the conclusion that the variability was due in some way to the animals and not to the extracts. Proof of this conclusion was obtained when we made three extracts and administered each in large equal doses (2 cc. daily for 5 days) to an immature rabbit and to an adult. In all these three experiments the adult uteri underwent full proliferation, while those of the immature animals underwent only partial proliferation. Because of this result we discontinued the use of immature animals for testing the corpus luteum extracts for their proliferative potency.

It should be added that 5 extracts of follicular fluid, which were made and tested on immature rabbits as controls for the above test, produced no specific proliferation of the endometrium, although they caused excellent general growth of the uterus (fig. 9), thus confirming the numerous workers quoted above who have obtained similar growth from various extracts of genital tissues. We are inclined to suppose, with Laqueur and his coworkers, that the general growth is due to the oestrin present in effective extracts. Since our corpus luteum extracts contain small amounts of oestrin, it is possible that the variable response of the endometrium is due to a varying degree of interaction between the effects of oestrin and the proliferative substance. In any case, the phenomenon of progestational proliferation is essentially an adult response and it is not surprising that uniform reactions are not obtained in infantile animals.

Previous work on endometrial proliferation. A number of previous workers have reported the preparation of potent extracts of the corpus luteum. Among these we may name Iscovesco (1912, 1914), Frank and Rosenbloom (1915), Fellner (1913), Seitz, Wintz and Fingerhut (1914), and Herrmann (1915). In all this work the test of potency used has been either the general growth-reaction, as evidenced by prevention of castrate atrophy or by hypertrophy of the immature uterus, or else an engorgement and swelling of the external genitals. In recent years, since the publication of E. Allen and Doisy's work, it has become evident that the effects used as tests of potency are associated with the action of the oestrin usually present in the corpus luteum extracts. This is of course confirmed by the

fact that in practically all the above-mentioned work the placenta has been found to yield extracts rivalling or exceeding the corpus luteum in potency. From this it has even been assumed by some that there is only one female sex hormone, and that the corpus luteum is negligible as a reproductive endocrine organ because it yields but little oestrin.

Examination of illustrations published by the workers named above indicates that the various extracts prepared by them from corpus luteum, placenta, and follicle fluid did not, in general, induce progestational proliferation of the uterus. We may cite Frank and Rosenbloom (1915, fig. 2—except perhaps cross-section 4); Schroeder and Goerbig (1921, figs. 2–5); Courrier and Potvin (1926); Laqueur, Borchardt, and de Jongh (1927, figs. 4–7). Herrman (1915) seems however to have elicited, in some of his experiments at least, the proliferation which our work indicates to be a specific effect of the corpus luteum. Figures 10 and 12 of his paper of 1915 reveal typical (though moderate) progestational proliferation, induced in the immature rabbit. He seems not to have grasped the full significance of this result, and in describing his experiments laid little emphasis upon the detailed histological reaction, using general growth and hyperemia of the genitalia as the important criteria of potency. He does not discriminate at all between the effects of extracts of corpus luteum and those of the placenta, while as we have mentioned, in our hands placental extracts have not given definite proliferation.

One of the figures published in 1913 by Fellner (fig. 2) taken together with the wording of his description, indicates that in some of his immature rabbits a state of proliferation was induced by an extract of the placenta, while in other experiments (compare his fig. 7) the uterus was not stimulated beyond the stage normal at mating. From his figures 15 and 16 it appears that Fellner's extracts of the corpus luteum did not induce progestational proliferation in the immature rabbit. One of the photographs presented by Frank and Rosenbloom (1915, fig. 2, section 4) hints at a slight degree of proliferation produced by a placental extract. To these three instances from the literature we may add that in one of 5 immature rabbits to which we administered extracts of human placenta made in the same way as our potent corpus luteum extracts, we elicited a slight and localized proliferation in part of the uterus. Steinach, Dohrn and colleagues (1928) seem to have produced something of the same kind in the young guinea pig, with placental extracts. We have already (p. 334) entered a reservation to the effect that the human placenta may possibly contain small amounts of the same active substance which is present in large quantity in the pig's corpus luteum. During the course of our work Hisaw (1927), Hisaw, Meyer and Weichert (1928) and Weichert (1928) published a series of preliminary notes describing the effects of extracts of the corpus luteum which they have found to produce certain phenomena

characteristic of pregnancy. Among these effects are relaxation of the symphysis pubis of the guinea pig, preparation of the endometrium to produce deciduomata, inhibition of ovulation, and alteration of the endometrium (rat) as in pregnancy. It seems likely that the active substance of these extracts is the same as that of ours, but we cannot be certain of this until we learn the method of preparation of Hisaw's extracts, about which nothing has been published except that they are made with acid alcohol. We look forward eagerly to the appearance of the definitive report on the methods and results of Hisaw and his fellow investigators.

It should be added that extracts very similar to ours have been prepared by Parkes and Bellerby (1927) who administered them to rats to determine their effect as regards inhibition of oestrus, but do not report an effect upon the endometrium. Payne, Van Peenan, and Cartland (1928) find that the unsaponifiable fraction of the corpus luteum lipoids acts to delay the return of oestrus in the guinea pig. We are not informed as to the chemical nature of the extracts used by Papanicolaou (1926) who also reports inhibition of oestrus. It is to be hoped that in the immediate future by the wider application of extracts of known potency in the production of one or another of these effects, investigators may achieve a complete understanding of the functions of the corpus luteum.

SUMMARY

The experiments described in this paper show that alcoholic extracts of the corpus luteum, freed from phospholipids, contain a substance which when injected into castrated adult female rabbits induces a characteristic alteration of the endometrium identical with the progestational proliferation previously shown to be due to the presence of corpora lutea in the ovaries. A similar effect is sometimes but not always produced in immature rabbits 8 to 12 weeks old. Extracts of follicular fluid containing large amounts of oestrin do not produce progestational proliferation, nor have extracts of human placenta given positive results. It appears, therefore, that the extracts of corpus luteum contain a special hormone which has for one of its functions the preparation of the uterus for reception of the embryos by inducing progestational proliferation of the endometrium.

We desire to express thanks to Parke, Davis & Company of Detroit and to Hynson, Westcott & Dunning of Baltimore for generous coöperation in the supply of ovarian extracts used in the earlier stages of this work.

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PHYSIOLOGY OF THE CORPUS LUTEUM

III. Normal Growth and Implantation of Embryos After Very Early Ablation of the Ovaries, Under the Influence of Extracts of the Corpus Luteum

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Received for publication December 19, 1928

In communications published in 1903 and 1910, Fraenkel demonstrated by a large series of experiments that removal of both ovaries of the rabbit, or destruction of all the corpora lutea, during the first 6 days after mating, always caused termination of the pregnancy. From these experiments Fraenkel concluded that the corpus luteum is an organ of internal secretion serving to facilitate implantation of the embryos. In the first paper of the present series (Corner, 1928) further proof of this hypothesis was given, by means of experimental removal of the ovaries, or by ablation of the corpora lutea, during the latter part of the first day after mating. In a series of 13 such experiments it was found that the embryos always degenerated on or before the 4th day of pregnancy, and that their death was correlated with a failure of the normal uterine reaction (progestational proliferation of the endometrium) by which presumably implantation is rendered possible.

In the rabbit implantation normally occurs on the 8th day. Since the embryos of does deprived of their corpora lutea do not survive beyond at most the 4th day, it is evident that the presence of lutein tissue is necessary not only for implantation, but also for nutrition or protection of the free blastocysts between the time of their arrival in the uterus early on the 4th day and implantation on the 8th day.

In the second paper of this series (Corner and Allen, 1929) we have given final proof of the causal relation between the corpus luteum and progestational proliferation of the uterus in the rabbit, by inducing typical proliferation in spayed does by means of extracts prepared from corpora lutea of swine. It remains only to determine whether the proliferation thus induced will serve to protect the embryos and bring about implantation. To answer this question we have recovered and studied the embryos of the 8 does which received effective doses of our corpus luteum extracts after ovulation and subsequent castration, with results shown in the following list.

X44. Six ruptured follicles. Autopsied $5\frac{1}{4}$ days after mating. One normal embryo 1.5 mm. in diameter; 1 degenerate, 1.5 mm., in uterus.

X46. Eleven ruptured follicles. At $5\frac{1}{4}$ days, 5 unsegmented degenerate ova in uterus.

X49. Eight ruptured follicles. At $5\frac{1}{4}$ days, 4 normal embryos, 1.25 to 1.6 mm.; 1 degenerate, 0.5 mm., 2 degenerating ova (fig. 2).

X55. Eight ruptured follicles. At $5\frac{3}{4}$ days, 1 normal embryo, 1.0 mm. (fig. 3).

X58. Nine ruptured follicles. At $5\frac{3}{4}$ days, 1 slightly retarded embryo, 1.1 mm.

X60. Eight ruptured follicles. At $5\frac{1}{4}$ days, 3 normal embryos, 1.25 to 1.75 mm.; 1 slightly retarded, 1.0 mm. (fig. 1).

X42. Thirteen ruptured follicles. At $6\frac{3}{4}$ days, 4 normal embryos, 2.5 to 2.9 mm., 2 slightly retarded, 1.75 mm.; 2 degenerate, 0.6 and 1.25 mm. (fig. 4).

X17. Eleven ruptured follicles. At $8\frac{1}{4}$ days, no embryos found.

Typical embryos are shown in the illustrations, figures 1 to 4. There are no exact standards by which to decide whether such blastocysts are normal or abnormal. The size can be taken as only a rough standard, since blastocysts of the 4th to the 8th day grow 100 per cent or more in diameter in 24 hours, and therefore a small variation in diameter is not significant. The embryos rated as normal in the above list all showed the characteristic texture of normal blastocysts and all bore distinct embryonic areas (inner cell masses). Judging from the findings in the larger litters 1.25 mm. is about the minimum normal diameter at $5\frac{3}{4}$ days, and 2.0 mm. the minimum normal diameter at $6\frac{3}{4}$ days. The blastocysts were found, in the various animals, in either cornu of the uterus; apparently the sectioning and ligature of the left cornu, done as a routine in each case, did not always prevent transportation of the embryos into the chamber above the ligature or necessarily hinder their growth.

In 6 of the foregoing 8 experiments, therefore, one or more of the embryos survived and continued to grow until the animal was killed on the 6th or 7th day of pregnancy, in spite of the removal of both ovaries of the mother at the 18th hour of pregnancy, and in spite of surgical trauma to the uterus involving resection of the middle portion of the left cornu.

This result led us to plan additional experiments to determine whether normal implantation could be induced by means of the corpus luteum extracts. Two does were chosen which had recently borne young but had lost their litters by refusal to nurse them. They had been isolated for about 40 days. Abstracts of the protocols follow.

X57. Mated November 13, 1928, at 5 p.m. Both ovaries removed November 14 at 11:30 a.m., $18\frac{1}{2}$ hours after mating. Eight freshly ruptured follicles in the ovaries. Care was taken to avoid all trauma to the uterus; a piece for histological control was therefore not excised, the history of the animal being guarantee that progestational proliferation was not present. One cubic centimeter of corpus luteum extract was administered daily throughout the experiment, beginning on the day of operation. On November 21, $7\frac{1}{4}$ days after mating, exploratory laparatomy dis-

closed swellings of the uterus, typical of 8 days' pregnancy. November 26 at 5:30 p.m., 13 days after mating, the animal was killed. The uterus presented 8 implantation sites about 18 mm. in diameter, as shown in the illustration (fig. 9), which contained normal embryos of 9 mm. crown-rump length (figs. 5, 6). Sections through several of the implantation-sites revealed perfectly normal conditions (fig. 5), and this was confirmed by microscopical examination. The stumps of the two ovarian pedicles, clearly located by the black silk ligatures, were serially sectioned and were found to contain no ovarian tissue.

X56. The experimental and operative procedures were identical with the above. Mated November 13 at 5 p.m., operation November 14 at 11 a.m. Eight freshly ruptured follicles were found in the ovaries. One cubic centimeter of the same extract was administered daily until 5 p.m., December 1, 17 days 21 hours after mating, when the animal was killed. At necropsy the uterus presented a normal pregnancy of the 18th day, as shown by the presence of 5 implantation sites about 30 mm. in external diameter (fig. 10), containing normal fetuses 22 mm. in length (crown-rump measurement), attached by normal placentas, as illustrated in figures 7, 8. Section through two of the implantation sites, confirmed by microscopic examination, revealed normal attachment of the placentas (fig. 8). The stumps of the ovarian pedicles were serially sectioned and found to contain no ovarian tissue.

The persistent growth and implantation of the embryos in the foregoing experiments may be contrasted with the fate of the embryos in 4 experimental groups serving as controls. We have already referred in our introductory paragraph to the first set of controls, reported in detail in the first paper of the present series (Corner, 1928) which show that under similar experimental conditions but without treatment of the mother with corpus luteum extracts, the embryos always die at about the 4th day. The second group of control observations comprises two animals which received injections of a potent corpus lutum extract, but in doses too small to elicit proliferation of the endometrium. In one of these two animals (both of which had of course ovulated and had been inseminated) no embryos or ova were found in the reproductive tract at necropsy; in the other (which had 11 ruptured follicles in the ovaries) 8 abnormal retarded blastocysts were

Fig. 1. Embryos of rabbit X60, $5\frac{1}{4}$ days old; three normal, one slightly retarded. ×7.

Fig. 2. Embryos of rabbit X49, $5\frac{1}{4}$ days old, showing 4 normal blastocysts and one degenerate. ×7.

Fig. 3. The one surviving embryo of rabbit X55, $5\frac{1}{4}$ days old. ×17.

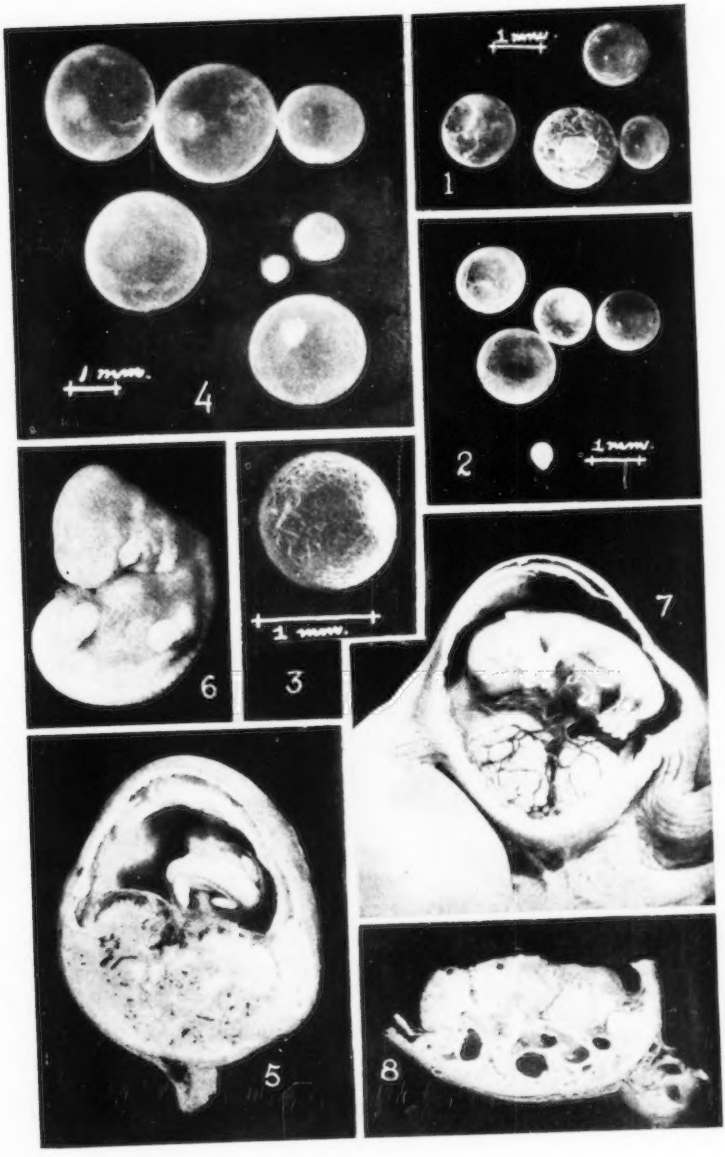
Fig. 4. Embryos of rabbit X42, $6\frac{1}{4}$ days old, showing 4 normal blastocysts, 1 slightly retarded, 2 degenerate. ×7.

Fig. 5. One of the implantation-sites of rabbit X57, showing normal placentation. ×2.5.

Fig. 6. One of the embryos of rabbit X57, 13 days old. ×4.

Fig. 7. One of the implantation-sites of rabbit X56, showing normal placentation and fetus 17 days, 21 hours old. ×1.5.

Fig. 8. Section through one of the placentas of rabbit X56, showing normal implantation. ×2.


1 mm.
1
1 mm.
4
2
1 mm.
6
1 mm.
3
7
5
8

found, the largest of them measuring 0.4 mm. in diameter. In this case the embryos had evidently survived only until the 4th day.

The third set of controls comprises 4 rabbits placed under similar experimental conditions, but treated with follicular extracts in dosage equivalent to 50 to 100 or more rat units in the 5 days' treatment. In the first of these, 7 ruptured follicles were found, and at necropsy 4 very early degenerated blastocysts of about the 3rd day were found in the two Fallopian tubes. In the second doe, 12 follicles had ruptured, and at necropsy one object taken to be a badly degenerated 8-cell embryo was found in the left uterine cornu above the ligature at the site of excision of the histological control specimen. In the third, 9 follicles ruptured, and as in the others, insemination had occurred, but in this case no embryos could be found in tubes or uterus at necropsy $5\frac{3}{4}$ days after mating. Thinking that perhaps the death of these embryos might be ascribable to abnormal conditions arising from the surgical trauma to the uterus rather than to the absence of proliferation, we studied a fourth experimental animal in which the excision of a sample of the uterine cornu was omitted. The doe was simply spayed 18 hours after mating, using care to avoid trauma to the uterus, and was then given 5 daily injections of a follicular extract. In this animal 7 follicles ruptured; at necropsy one ovum was found in the left Fallopian tube, so far degenerated that it could not be certainly determined whether or not segmentation had occurred. At any event this ovum had not survived longer than the second day.

The fourth group of controls comprises 2 does treated with an extract of human placenta. In the first animal 9 follicles ruptured; she received in 5 days a quantity of the extract containing 400 rat units of oestrin. At necropsy no ova were found. In the second animal trauma to the uterus was again avoided. In this doe 11 follicles ruptured; she was given 100 rat units of the extract; at necropsy no ova were found.

As described in the preceding paper (Corner and Allen, 1929) none of these control animals underwent progestational proliferation. It is obvious that large doses of oestrin do not protect the embryos against the early degeneration which is brought on by removal of the ovaries. It seems certain, indeed, that the injection of oestrin in some way retards at a very early stage either the growth or the transportation of the embryos, or both, for in these control experiments with extracts containing oestrin the embryos had either disappeared entirely or were found in advanced degeneration in the

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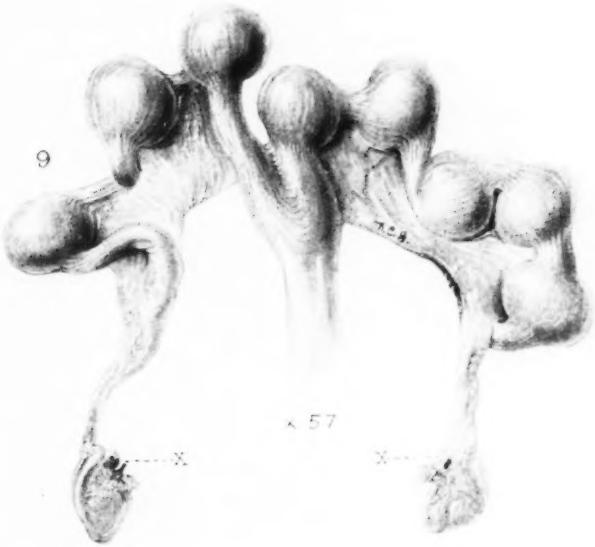
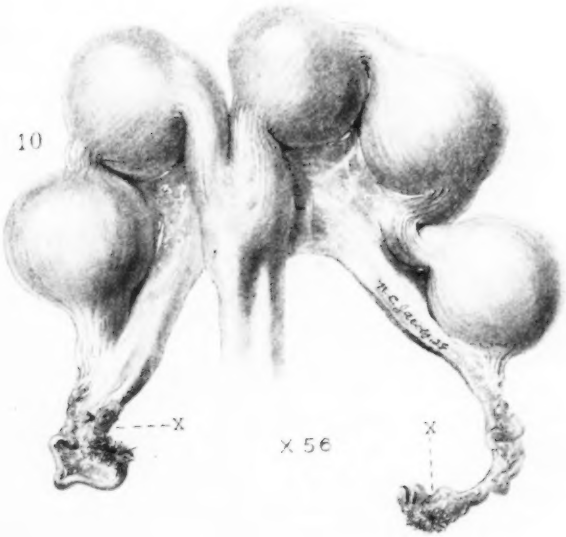
Fig. 9. Drawing of pregnant uterus of rabbit X57. $\times\frac{1}{2}$. The letter *x* indicates site of excision of ovaries.

Fig. 10. Drawing of pregnant uterus of rabbit X56. $\times\frac{1}{2}$. The letter *x* indicates site of excision of ovaries.


9
x 57
X
X


10
X
X
X 56

Fallopian tube at a time when in spayed untreated animals they should have been found in the uterus as blastocysts .2–.4 mm. in diameter.

We do not as yet understand the mechanism of this remarkably early destruction of the embryos by oestrin in the absence of the ovaries. A similar effect may be responsible for the fact discovered by Margaret Smith (1926) that in the rat pregnancy is always interrupted if oestrin be administered during the first five days.

SUMMARY

These experiments demonstrate that in the presence of progestational proliferation induced by corpus luteum extracts, in rabbits deprived of both ovaries at the 18th hour of pregnancy, the embryos may survive and grow normally and normal implantation may occur, whereas in the absence of progestational proliferation the embryos never survive beyond the fourth day. The evidence is now complete that in the rabbit the corpus luteum is an organ of internal secretion which has for one of its functions the production of a special state of the uterine mucosa (progestational proliferation) and that in turn the function of the proliferated endometrium is to nourish or protect the free blastocysts and to make possible their implantation.

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THE EFFECT OF THE RATE OF STIMULATION, STRENGTH OF STIMULUS, SUMMATION AND REËNFORCEMENT ON THE RATE OF THE CONDUCTION OF A NERVE IMPULSE THROUGH REFLEX ARCS

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Received for publication January 7, 1929

It is generally accepted that the rate of stimulation, the strength of the stimulus, summation and reënforcement are factors which have a very material effect upon the extent of the responses in the deep reflexes. It was suggested that these same factors might alter conduction time. In order to demonstrate the truth or falsity of this idea the present investigation was undertaken.

Before going further with the discussion of the present problem the writer wishes to set forth just what he means by "conduction time." This term has been arbitrarily adopted to represent the interval of time elapsing between the application of a stimulus to a receptor and the change in the electrical potential at a muscle as indicated by the action current.

The data were collected by means of a special apparatus devised by Travis and Hunter (1928). Briefly it consists of a three-stage resistance-coupled amplifier, a three-element oscillograph with photographic equipment, a vacuum-tube oscillator, a signal circuit and an automatic stimulator. The arrangement and construction of the oscillograph, oscillator and signal circuit is too complicated for detailed discussion here. The same technique was used as described by Travis and Hunter, except the electrodes were placed over the gastrocnemius instead of the rectus femoris muscle.

The reflex arc involved in the Achilles-jerk is used as a basis for this study. Figure 1 is a representative picture of the conduction rate through this arc.

The dip in line *C* (fig. 1) indicates the time of the application of the stimulus; the disturbance, *1*, in line *A* is due to the spark gap between the stimulating hammer and the copper plate over the Achilles tendon and need not be considered in reading the record. Disturbance *2* in line *A* shows the arrival of the action current at the gastrocnemius muscle. Line B represents time in $\frac{1}{100}$ second.

Six pictures were taken for each experimental condition in each subject.

The effect of the rate of stimulation on the rate of conduction of a nerve impulse through a reflex arc. Data were collected from four normal male subjects. The stimuli were delivered at the rate of 12, 6, and 2 per minute. The data are shown in table 1.

For subject 1 the conduction time was found to be 0.042 second, regardless of the rate of stimulation; for subject 2 it was 0.040 second; for 3, 0.033 second; and for 4, 0.032 second. These figures each represent the average of six records. The deviation from the average in each case was not more than $\frac{1}{1000}$ second.

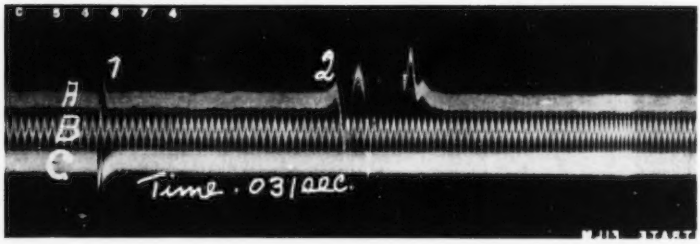

Fig. 1. A record of the conduction time through the reflex arc involved in the Achilles-jerk.

TABLE 1

The effect of the rate of stimulation on the rate of conduction of a nerve impulse through a reflex arc

SUBJECT	RATE OF CONDUCTION		
	5 second intervals	10 second intervals	30 second intervals
	seconds	*seconds*	*seconds*
1	0.042	0.042	0.042
2	0.040	0.040	0.040
3	0.033	0.033	0.033
4	0.032	0.032	0.032

TABLE 2

The effect of the intensity of the stimulus on the rate of conduction of a nerve impulse through a reflex arc

SUBJECT	RATE OF CONDUCTION			
	Intensity 1	Intensity 2	Intensity 3	Intensity 4
1	0.041	0.041	0.041	0.041
2	0.040	0.040	0.039	0.040
3	0.033	0.033	0.033	0.033
4	0.032	0.032	0.032	0.032

The effect of the intensity of the stimulus on the rate of conduction of a nerve impulse through a reflex arc. Data were collected from the same subjects used in the preceding experiment. Four different intensities of stimulation designated as intensities 1, 2, 3 and 4, were delivered to the Achilles tendon

at a uniform rate of six per minute. Although the exact equivalent in grams was not determined for the different stimuli, they represent strengths, one which is just adequate, one which is maximal, and two which are of different intermediate intensities. The data are shown in table 2.

For subject 1 the conduction time is 0.041 second, regardless of the strength of stimuli used; for 2 it is 0.040 second; for 3, 0.033; and for 4, 0.032. The deviation from the average of the six pictures taken for each subject for every intensity was not more than $\frac{1}{100}$ second.

TABLE 3

The effect of reënforcement on the rate of conduction of a nerve impulse through a reflex arc

RECORD	RATE OF CONDUCTION			
	Subject 1	Subject 2	Subject 3	Subject 4
	second	*second*	*second*	*second*
1	0.042	0.039	0.033	0.031
2	0.042	0.040	0.033	0.032
3	0.042	0.037	0.033	0.032
4	0.042	0.040	0.033	0.032
5	0.042	0.039	0.033	0.032
6	0.042	0.038	0.033	0.032
Average.....	0.042	0.039	0.033	0.032

TABLE 4

The effect of summation on the rate of conduction of a nerve impulse through a reflex arc

RECORD	RATE OF CONDUCTION			
	Subject 1	Subject 2	Subject 3	Subject 4
	second	*second*	*second*	*second*
1	0.042	0.039	0.034	0.032
2	0.042	0.039	0.033	0.031
3	0.042	0.041	0.033	0.032
4	0.042	0.041	0.034	0.032
5	0.042	0.040	0.034	0.032
6	0.042	0.041	0.034	0.032
Average.....	0.042	0.040	0.034	0.032

The effect of reënforcement on the rate of conduction of a nerve impulse through a reflex arc. As in the previous experiments four subjects were used. Just before the delivery of the stimulus the subject was instructed to clinch either his hands or teeth when the signal was given. In each case the observations of the experimenter varified the effectiveness of the reënforcing agent. Stimuli of uniform strength were delivered at the rate of six per minute. The data are shown in table 3.

The time interval as indicated by each of the six pictures taken is shown in table 3. In case of subject 1 the average conduction time is 0.042 second; for 2, 0.039 second; for 3, 0.033 second; for 4, 0.032 second. In the records for subjects 1 and 3, there is no deviation from the average. In no case is the deviation more than 2/1000 second, this occurring in but one case.

The effect of summation on the rate of conduction of a nerve impulse through a reflex arc. Here again four subjects were used. The experimenter undertook to establish a summation effect by delivering twenty-one stimuli at a rapid rate, the twenty-first being recorded. The object of the experiment was an attempt to modify synaptic resistance by rapidly repeated stimulation. The readings of all the pictures taken of each subject are shown in table 4.

TABLE 5

The constancy of conduction time under various experimental conditions

SUBJECT	RATE OF CONDUCTION			
	With all rates stimulation	With all strength stimulation	Summation	Reënforcement
	second	*second*	*second*	*second*
1	0.042	0.041	0.042	0.042
2	0.040	0.040	0.039	0.040
3	0.033	0.033	0.033	0.034
4	0.032	0.032	0.032	0.032

The average conduction time for subject 1 is 0.042 second with no deviation from the average. For subject 2 it is 0.040 second; for 3, 0.034 second; for 4, 0.032 second. In no case is the deviation greater than $\frac{1}{1000}$ second.

CONCLUSIONS

Data were collected from four subjects in an attempt to show the effect of the rate of stimulation, strength of stimuli, summation and reënforcement on conduction time involved in the Achilles-jerk.

The data in table 5 show that these factors have no effect on the conduction time of the subjects used in this experiment.

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OBSERVATIONS ON THE FUNCTION OF THE GALL BLADDER

Experiments with Methylene Blue on Rabbits

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Received for publication December 22, 1928

The ingenious invention by Graham and Cole (1) of a method of rendering the gall bladder content opaque to the Roentgen rays opened a new field for the study of the function of this organ and quickly became a most valuable addition to the list of indispensable methods of clinical diagnosis. About the beginning of 1924, when cholecystography was still in the hands of the discoverers and indeed in an early experimental stage, suggestions had been made as to the function of the gall bladder which were radically unlike the current views. Sweet (2), then in Philadelphia, and Halpert (3) in Prague and Frankfurt am Main pointed out simultaneously and independently that the gall bladder can hardly be looked upon as a reservoir the function of which is the supplying of concentrated bile whenever there is call for such in the intestine. They concluded that the bile which once entered the gall bladder did not leave it again, under ordinary conditions, through the cystic duct, but was resorbed by the mucous membrane of the biliary vesicle, and the constituents were then returned by the way of the veins and lymphatics into the liver and into the general circulation respectively. These views, based upon morphological data, experimental and clinical observations of their own and of many others before them, seemed suited to solve many of the riddles encountered in the normal anatomy and physiology as well as in the pathology of the gall bladder and bile ducts and were proposed for that reason. But since no conclusive evidence could be furnished at that time, they were offered rather as a working hypothesis than a rigid doctrine.

This proposal seemed to many so unlikely that they passed over it while others set out to disprove it. Cholecystography just then discovered and developing rapidly, seemed fit to serve the purpose. It would lead, however, too far a field to review and it would be too early to evaluate the wealth of work accomplished since cholecystography became a routine clinical procedure (4). Nevertheless, it may be said that in spite of the refined technique in producing shadows and estimating the amount of the content of the gall bladder (5) most of the problems in regard to the function of this organ are still under active discussion (6), (7), (8). For our

part we fully agree with Blond (9), (10), who emphasized recently that the interpretation of the results of cholecystography is easier and the findings less contradictory if it is assumed that the bile enters the gall bladder, not to be stored there, and expelled in time, but to be resorbed in toto by the gall bladder mucosa. The fact that bile may and does leave the gall bladder occasionally in small quantities does not invalidate this conception, nor do the experimental results of Boyden (11), Whitaker (12), (13), Higgins and Mann (14), Hamrick (15), Ivy and Oldberg (16), and of others make it untenable. It has been shown by these authors that the gall bladder can be made to expel some of its content and they have demonstrated the actual passage of certain opaque substances from the biliary vesicle into the duodenum. This, however, is far from proving that the gall bladder empties most of its content through the cystic duct under ordinary conditions or that its function is to do so. The anatomical arrangement about the neck of the gall bladder and in the cystic duct, regulating, as it does, inflow and hindering or preventing outflow, shows, to be sure, a wide enough range of variations, from perfect or nearly perfect competency (especially in children) to total derangement, mainly in conditions of biliary stasis, to account for a great deal of their experimental results.

An incidental observation made in Doctor Petroff's laboratory in the Trudeau Sanatorium gave new impetus to the search for conclusive evidence for or against the new hypothesis of the function of the gall bladder. Doctor Petroff treated his colony of rhesus monkeys, afflicted with intestinal parasites, orally with methylene blue. At necropsy of one of these monkeys several days after the last dose, methylene blue was discovered in the gall bladder. Thus methylene blue seemed a promising agent for determining the correctness of the assumption that the bile which once entered the gall bladder does not leave it again under ordinary conditions through the cystic duct. Moreover, the bactericidal propertes of the dye suggested the possibility of its use as a biliary antiseptic for therapeutic purposes. From preliminary experiments on rabbits and guinea pigs, it was soon learned, however, that not all of the methylene blue excreted in the bile appared there as such but much of it was present in a so-called leuko-form, and this leuko-form has no definite bactericidal effect in vitro on a number of microörganisms tested (Staph. albus, Staph. aureus, Streptococcus viridans, etc.). These in vitro experiments, of course, do not exclude the possibility of methylene blue being effective in vivo. It was therefore decided to carry out further investigation in this direction after sufficient data have been collected on the mechanism of the excretion of methylene blue. Simultaneously search is in progress for a substance which combines bactericidal properties with opacity to Roentgen rays, yet behaves like methylene blue, i.e., appears in the bile and accumulates in the gall bladder when it is administered intravenously or by mouth.

In this paper we are presenting the data obtained in a series of experiments with methylene blue on rabbits. The information gained proves beyond doubt that at least in this animal when bile leaves the gall bladder through the cystic duct, this is rather an exception than the rule. The experiments also illustrate the possibility of a substance appearing in the bile and being retained in the gall bladder for a considerable period of time, which in turn, we believe, throws some light upon the mechanism of the formation of biliary concretions (17), (18).

METHOD. The main difficulty in obtaining accurate data on the mechanism of the excretion of methylene blue by the biliary system lay in the lack of a method for the quantitative determination of methylene blue in bile. After many unsuccessful attempts the following method devised by one of us (M.T.H.) proved to be reliable and accurate:

Mix 1 cc. bile, 1 cc. 30 per cent lead acetate, 1 cc. water, and 0.2 cc. of a 10 per cent solution of sodium persulphate. Add 6.8 cc. 95 per cent alcohol. Centrifuge after 2 hours. Compare the clear supernatant fluid in a colorimeter with a standard 1:100,000 methylene blue solution containing all the constituents used in the above reaction, excepting bile and persulphate.

Lead acetate was used to precipitate the bile pigments. This precipitation is quantitative. The lead precipitate adsorbs a considerable amount of methylene blue from an aqueous solution; the dye, however, is not adsorbed by the lead precipitate in 60 to 70 per cent alcohol solutions. Since a considerable part of the methylene blue appeared in the bile in the so-called leuko-form, it was necessary to oxidize it in order to regenerate the color: sodium persulphate was used to effect this oxidation.

When the above procedure is carried out on normal bile, a brownish grey precipitate of lead salts is obtained, the supernatant liquid being colorless. Bile that contains methylene blue gives a similar brownish grey precipitate and a blue supernatant liquid.

Attention should be called to the fact that the bile is diluted ten times. The usual standard is prepared by diluting 1 cc. of a 1:10,000 solution of methylene blue to 10 cc. as previously described. The comparison is really carried out against a 1:100,000 standard. Some of the bile samples contained so little methylene blue that a 1:1,000,000 standard had to be used for comparison. The color is intense enough, even at this extreme dilution, to give perfectly reliable figures.

In all of the experiments, which we are presenting here, methylene blue was administered to rabbits in doses of 20 mgm. per kilogram body weight, i.e., 2 cc. of a 1 per cent solution, in one series intravenously, in another by stomach tube. The bile was collected in 1 cc. portions, the rate of flow recorded and the methylene blue content determined by the above method.

INJECTION EXPERIMENTS. In the injection series, the animal was

anesthetized with ether, shaved on the neck, abdomen and right ear, a glass cannula tied into the trachea, and the animal allowed to breathe through this from an ether bottle. The abdomen was then opened and a glass cannula tied into the ductus choledochus close to its termination into the duodenum. A short rubber tube connected this with a graduated pipette of 1 cc. capacity. After one or two 1 cc. samples of bile had been collected, the amount of methylene blue calculated for the weight of the animal (2 cc. of a 1 per cent solution of methylene blue per kilogram body weight) was injected into the marginal vein of the right ear. The time that

TABLE 1

Injection experiments

Summary of data on the excretion of methylene blue by the biliary system of the rabbit after intravenous administration of the dye (2 cc. of a 1 per cent solution per kilogram body weight).

RABBIT	FIRST APPEARANCE OF DYE IN THE BILE	HIGHEST CONCENTRATION OF DYE IN THE BILE FROM D. CHOLEDOCHUS				DURATION OF EXPERIMENT	AVERAGE FLOW OF BILE PER HOUR	CONCENTRATION OF DYE IN BILE AT CLOSE OF EXPERIMENT		
		Reached within	Dilution	Approximately maintained for				From D. choledochus	From gall bladder	Ratio of concentration (approximately)
	minutes	*minutes*		*hrs.*	*minutes*	*hrs.*	*cc.*			
I 8	6	42	1:1,160	1		2	8	1:1,460	1:1,460	1:1
I 15	8	28	1:3,000	1	30	2	5	1:4,000	1:1,200	1:3
I 24	5	40	1:3,200	1		3	4	1:4,040	1:1,680	1:2
I 16	8	71	1:1,070	2	30	6	5.5	1:9, 000	1:730	1:12
I 23	3	33	1:1,600	1	30	6	8	1:28,000	1:1,470	1:19
I 17	4	45	1:2,000	1	30	6	10	1:9, 800	1:800	1:12
I 25	4	16	1:2,050		30	6	13	1:26,000	1:16,800	1:1.6
I 26	4	31	1:2,250	1	15	6	6	1:11,800	1:2,480	1:4.7
I 10	10	26	1:2,400	1	30	6	10	1:16,000	1:720	1:22
I 20	4	21	1:2,500		35	6	8.5	1:36,000	1:3,000	1:12
I 27	5	30	1:3,270		45	6	6.5	1:10,500	1:4,130	1:2.5
I 14	15	30	1:1,600		45	7	11	1:12,000	1:1,800	1:7

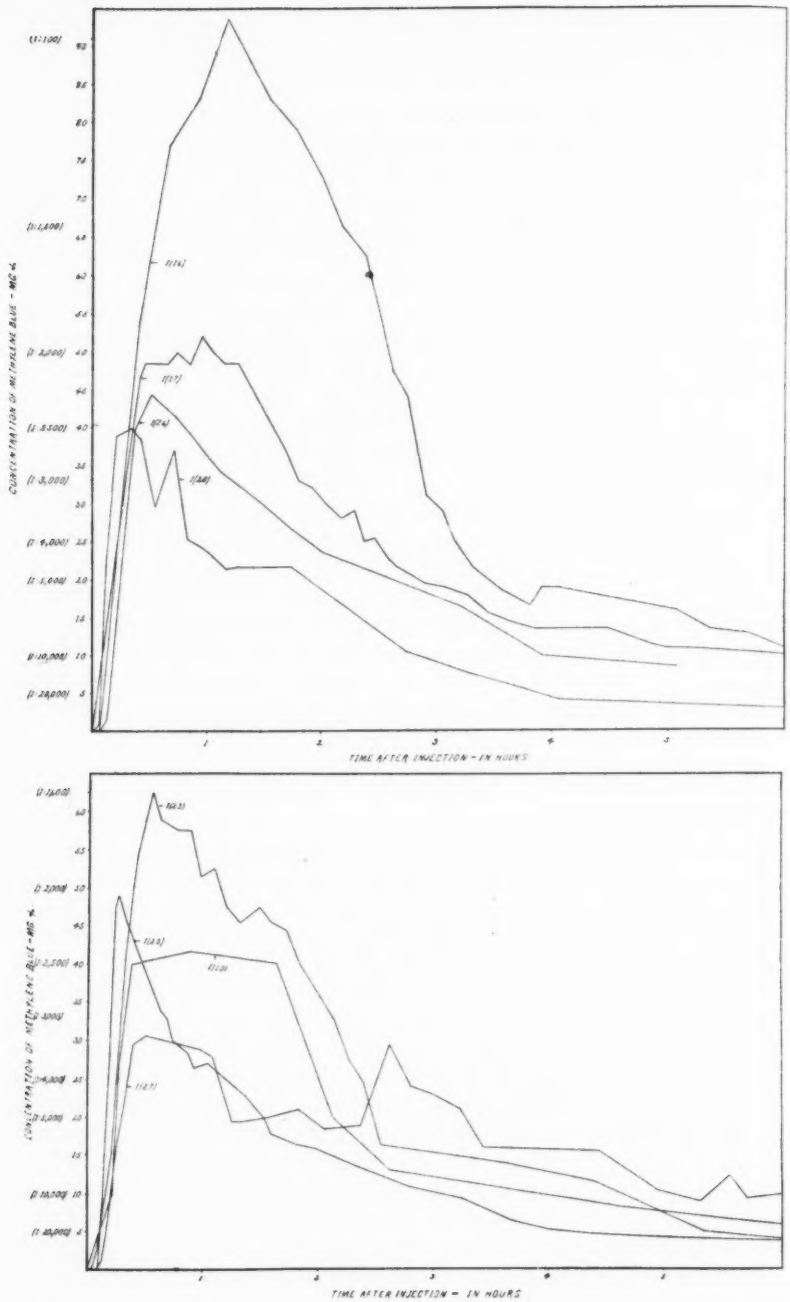
elapsed between the injection and the gross appearance of the dye in the pipette was noted, the bile was collected in 1 cc. portions, the rate of flow recorded and the methylene blue content determined.

The results obtained are charted in table 1 and may be summarized briefly as follows: 1. Injected intravenously, methylene blue appeared in the bile in from three to fifteen minutes. 2. The highest concentration of the dye in the bile collected from the ductus choledochus was usually reached within twenty to forty-five minutes, but in one instance only sixteen and in another seventy-one minutes were necessary. 3. The length

of time during which this maximum concentration—1:1,100 to 1:3,300—was approximately maintained varied between one and two hours. In one instance, however, it lasted for two and a half hours and in some only for about half an hour, after which it decreased gradually. 4. Values ranging between 1:9,000 and 1:36,000 were obtained six hours after the injection. 5. The bile removed from the gall bladder at the same time, i.e., six hours after the injection, usually contained two to twenty-two times as much methylene blue as that contained in the last sample of bile obtained from the ductus choledochus. Not only that but in three out of eight cases the concentration of methylene blue in the bile removed from the gall bladder was much higher—1:720, 1:800—than the highest concentration ever reached in the bile collected from the ductus choledochus.

This latter observation was the more remarkable since the gall bladder was never empty to begin with and since during the entire period of the experiment bile was passing through the cannulated ductus choledochus at a rate of 5.5 to 13 cc. per hour. In explaining these findings it was necessary to assume that either bile had passed through the cystic duct into the gall bladder irrespective of the cannula in the ductus choledochus and that in the biliary vesicle a rapid concentration of the dye had taken place, or that the main bulk of the methylene blue reached the gall bladder by the way of the blood stream and was excreted by its mucous membrane. Mann's experiments with rose bengal (19) supported this latter assumption. In order to clear the situation, the cystic duct was ligated in two rabbits, the usual amount of methylene blue injected intravenously and the bile removed from the gall bladder two hours later. The estimated content of the biliary vesicle was about 2 or 3 cc. at the time of the ligation and injection. About two hours later less than 0.2 cc. of bile could be obtained from the gall bladder, which contained a hardly determinable trace of methylene blue. Obviously then the gall bladder mucosa was not excreting the dye. In other words, the methylene blue found in the gall bladder in the experiments of this investigation must have entered the viscus via the cystic duct.

In figures 1 and 2 it may be observed that the curve indicating the methylene blue content of the bile coming down from the liver was a very regular one: a sharp rise to the peak of a highest concentration was followed by a gradual decline. On the other hand, during the whole period of this decline and up to the end of the experiment, the methylene blue content of the bile in the gall bladder increased and at the end of the experiment was usually found to be many times higher than that coming down from the liver. Had there been an occasional admixture of the bile from the gall bladder, with its high content of methylene blue, and that coming from the liver, the curve of declining methylene blue concentration in the ductus choledochus would have exhibited irregularities. Inasmuch, however, as the curve showing the methylene blue content of the bile collected from the cannulated ductus



Figs. 1 and 2. Graphical presentation of the methylene blue content of the bile collected from the ductus choledochus—after intravenous administration of the dye—during an experimental period of about six hours.

choledochus remained regular, it may be assumed that no bile left the gall bladder.

FEEDING EXPERIMENTS. In this series of experiments the methylene blue was given by stomach tube. The amount administered was exactly the same as in the injection experiments and the operative procedure was also identical. After the administration of the dye the animals remained in their usual environment and on their usual diet until the time of operation, particular care being taken that there always was food in their cages. The operation was performed at certain stipulated intervals between the 12th and 72nd hour after the ingestion of the dye in order to allow time enough for the action of any physiological process which would tend to free the gall bladder from its methylene blue content.

The results obtained in these experiments are charted in table 2. From these we learn that bile collected from the cannulated ductus choledochus 12, 18, 24, 30 and 36 hours after administration of methylene blue by stomach tube contained little or no methylene blue. Bile removed from the gall bladder at the same time, on the other hand, contained the dye invariably. Bile collected from the cannulated ductus choledochus 42, 48, 60 and 72 hours after administration of methylene blue by stomach tube, contained no methylene blue. Again, bile removed from the gall bladder on the other hand contained the dye in most instances even after 72 hours.

Examining more closely the data on the feeding experiments we note that the bile collected from the ductus choledochus never contained methylene blue in a higher concentration than 1:23,000 in any of the 34 animals which have been opened between the 12th and the 36th hours after feeding of the dye. In 14 the bile contained no methylene blue at all. Yet the bile in the gall bladder of these aminals contained the dye in concentrations between 1:1,200 and 1:10,000 in 19 cases, and between 1:10,000 and 1:25,000 in 11 cases. The results in the 42, 48, 60 and 72 hour groups of 32 animals are quite uniform. As mentioned before, in none of the animals did the bile collected from the cannulated ductus choledochus contain any methylene blue. Yet the bile in the gall bladder at this time contained the dye in more than half of the cases in concentrations ranging between 1:4,850 and 1:26,000.

DISCUSSION. The data obtained from the study of the excretion of methylene blue by the biliary system of the rabbit furnish a great deal of information concerning the mechanism of the function of the gall bladder.

Methylene blue injected intravenously appeared in the bile in a few minutes and reached a highest concentration—1:1,100 to 1:3,300—within less than one hour. This concentration was maintained for an hour or two; then, dropping gradually, it showed from a fifth to a fifteenth of the highest concentration by the end of the sixth hour, the values ranging between 1:9,000 and 1:36,000. The bile removed from the gall bladder at the same

TABLE 2

Feeding experiments

Concentration of methylene blue in the bile collected from the cannulated ductus choledochus and in the bile removed from the gall bladder at certain stipulated intervals between the 12th and the 72nd hour after oral administration of the dye (2 cc. of a 1 per cent solution per kilogram body weight).

RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE
12-hour group			
F-52	1:28,000	1:5,400	1:24,000
F-75	1:80,000	1:5,490	1:43,600
F-51	1:27,700	1:7,100	1:4,650
F-78	1:28,400	1:8,200	1:15,200
F-79	1:31,000	1:14,000	Trace
F-80	1:100,000	1:25,000	1:30,000
18-hour group			
F-45	1:30,000	1:1,480	1:4,750
F-9	None	1:3,700	1:28,000
F-44	1:43,000	1:6,200	1:19,200
F-41	None	1:10,500	1:62,500
F-5	1:80,000	1:20,000	
F-22	None	1:24,000	Trace
24-hour group			
F-30		1:1,200	1:10,000
F-39	1:60,000	1:2,070	
F-35	1:23,000	1:4,250	1:3,000
F-17	1:30,000	1:5,000	1,6:500
F-29	1:42,000	1:5,300	1:70,000
F-40	1:70,000	1:6,200	Trace
F-47	None	1:6,580	
F-36	None	1:7,400	None
F-10		1:9,000	None
F-13	1:55,000	1:13,400	1:60,000
30-hour group			
F-72	Trace	1:3,960	Trace
F-74	None	1:9,720	None
F-70	1:76,000	1:11,160	1:17,600
F-18	None	1:14,000	None
F-69	None	1:24,600	Trace
F-7	None	1:25,000	None
36-hour group			
F-53	1:35,000	1:1,750	1:5,400
F-71	None	1:22,000	None
F-54	None	1:37,000	
F-73	None	1:57,000	None
F-77	None	Trace	None
F-76	None	Trace	None
42-hour group			
F-43	None	1:5,500	None
F-14	None	1:6,100	None
F-46	None	1:15,500	None
F-42	None	1:21,500	None
F-59	None	1:40,000	None
F-58	None	1:100,000	None
48-hour group			
F-31	None	1:4,850	None
F-49	None	1:7,500	None
F-26	None	1:10,000	None
F-4	None	1:12,000	None
F-37	None	1:22,000	None
F-50	None	1:26,000	None
F-32	None	1:50,000	None
F-60	None	1:60,000	None
F-38	None	1:100,000	None
F-61	None	Trace	None
60-hour group			
F-66	None	1:7,400	None
F-57	None	1:12,300	None
F-56	None	1:21,000	None
F-63	None	1:25,000	None
F-55	None	1:43,000	None
F-62	None	Trace	None
72-hour group			
F-33	None	1:6,000	None
F-67	None	1:13,870	None
F-64	None	1:15,300	None
F-68	None	1:21,600	None
F-3	None	1:26,000	None
F-65	None	1:81,000	None
F-34	None	Trace	None
F-15	None	None	None
F-19	None	None	None
F-20	None	None	None

time, i.e., six hours after the injection contained two to twenty-two times as much of the dye as the last sample of bile collected from the ductus choledochus. At times, moreover, the concentration of the dye in the bile removed from the gall bladder was much higher—1:720, 1:800—than the hightest concentration ever reached in the bile collected from the ductus choledochus, although the gall bladder was never empty to begin with and bile was passing freely through the cannulated ductus choledochus at a rate of 5.5 to 13 cc. per hour.

An additional and noteworthy feature was the fact that in all of these experiments the bile column in the pipette connected with the cannulated ductus choledochus moved forward only during inspiration. From this it appears safe to assume that the pressure exerted upon the liver during inspiration is the main force driving the bile into the gall bladder.

In the feeding experiments the methylene blue was introduced through a catheter into the stomach; consequently the greater bulk of the dye which reached the liver must have come there by the way of the portal vein. We have no information as to how soon and in what initial concentration the dye appears in the bile after oral administration and how long it takes to reach the highest concentration and what this highest concentration is. But we know that in none of the 22 animals opened between the 12th and the 24th hours after feeding of the dye did the bile collected from the ductus choledochus contain methylene blue in a higher concentration than 1:23,000, although the bile in the gall bladder contained the dye in concentrations between 1:1,200 and 1:10,000 in 16 cases. Furthermore out of the 12 animals which have been opened between the 30th and the 36th hours after feeding of methylene blue there were only two in which the bile collected from the ductus choledochus contained any of the dye. Out of the 32 animals which have been opened between the 42nd and the 72nd hours after feeding of methylene blue and in which the bile collected from the ductus choledochus contained no methylene blue, the bile removed from the gall bladder contained the dye in more than half of the cases in concentrations ranging between 1:4,850 and 1:26,000.

From the feeding experiments we learned that ingested methylene blue appears in the bile, is excreted for some time, during which the pure bile contained in the gall bladder is gradually replaced by the dye containing bile. Since the gall bladder mucosa is apparently resorbing the dye less rapidly than it resorbs bile, the dye accumulates and stays there. Attention should be called to the fact that the amount of bile delivered through the cannulated ductus choledochus during an experimental period of six hours averages about 10 cc. per hour, i.e., the liver of a 3 kgm. rabbit produces, conservatively estimated, 240 cc. of bile per day; yet the average capacity of the rabbit's gall bladder is only about 3 cc. Furthermore in the feeding experiments, as mentioned before, the animals were kept on their

usual diet and there was always food in their cages. Under these conditions it is hard to conceive how methylene blue could remain in the gall bladder of an animal for a period of at least 42 hours during which at least 420 cc. of bile not containing the dye could have come from the liver, unless we give up the idea that the function of the gall bladder is to empty and to refill. In the face of all the evidence, just presented, we feel safe to conclude that at least in the rabbit when bile leaves the gall bladder through the cystic duct this is rather an exception than the rule.

SUMMARY

1. Methylene blue administered intravenously or by mouth appears in the bile. This fact was utilized to obtain information concerning the mechanism of the function of the gall bladder.

2. A colorimetric method is described by which it is possible to determine methylene blue accurately in bile.

3. Injected intravenously (2 cc. of a 1 per cent solution per kilogram body weight), methylene blue appears in the bile collected from the ductus choledochus within a few minutes. A sharp rise to the peak of a highest concentration (1:1,100 to 1:3,300) reached approximately within one hour, is followed by a gradual decline to from a fifth to a fifteenth of the highest concentration (1:9,000 and 1:36,000) by the end of the sixth hour.

4. Bile removed from the gall bladder six hours after injection, contains two to twenty-two times as much of the dye as the last sample of bile collected from the ductus choledochus.

5. The concentration of the dye in the bile removed from the gall bladder is at times much higher (1:720, 1:800) than the highest concentration ever reached in the bile coming from the liver (1:1,100—1:3,300).

6. The gall bladder mucosa does not excrete the dye: all the methylene blue reaches the gall bladder via the cystic duct.

7. The pressure exerted upon the liver during inspiration is the main force driving the bile into the gall bladder.

8. Bile collected from the cannulated ductus choledochus between the 12th and the 72nd hour after feeding the dye, contained little or no methylene blue. Bile removed from the gall bladder contained the dye in most instances even after 72 hours.

9. The explanation offered for the presence of methylene blue in the gall bladder long after the liver ceased to produce bile which contains the dye, is as follows: a, the gall bladder resorbs methylene blue and its leukoforms much more slowly than it resorbs bile, and b, bile does not under ordinary conditions leave the gall bladder through the cystic duct.

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